EXPERIMENTAL DIABETES

AND ITS RELATION TO THE CLINICAL DISEASE

A SYMPOSIUM

organized by

THE COUNCIL FOR INTERNATIONAL ORGANIZATIONS OF MEDICAL SCIENCES

Established under the joint auspices of UNESCO *and* WHO

Consulting Editors

J P Hoet (Belgium)

F G Young (U K)

Editors for the Council

J F Delafresnaye
C I O M S ,
Paris, France

G Howard Smith
Department of Biochemistry
Cambridge, England

BLACKWELL SCIENTIFIC PUBLICATIONS
OXFORD

Published simultaneously in the United States of America by Charles C Thomas, Publisher, 301-327 East Lawrence Avenue, Springfield, Illinois

Published simultaneously in Canada by The Ryerson Press, Queen Street West, Toronto 2

FIRST PRINTED JANUARY 1954

PRINTED IN GREAT BRITAIN IN THE CITY OF OXFORD
AT THE ALDEN PRESS

LIST OF PARTICIPANTS

Charles H Best	Toronto, Canada
James Campbell	Toronto, Canada
J L R-Candela	Madrid Spain
Jerome W Conn	*St Louis Missouri, U S A*
Carl F Cori	St Louis, Missouri, U S A
Gerty T Cori	Ann Arbor Michigan, U S A
Christian de Duve	Louvain Belgium
J H Gaarenstroom	Groningen, The Netherlands
Mervyn Griffiths	Canberra Australia
H C Hagedorn	Gentofte, Denmark
Per Hanssen	Stavanger, Norway
*J P Hoet	Louvain, Belgium
S E de Jongh	Leiden The Netherlands
R D Lawrence	London, England
Arnold Lazarow	Cleveland Ohio, U S A.
C N H Long	New Haven, Connecticut, U S A
A Loubatieres	Montpellier, France
F D W Lukens	Philadelphia, Pennsylvania, U S A
Jane A Russell	Emory, Georgia, U S A
F Verzár	Basel, Switzerland
A E Wilhelmi	Emory, Georgia, U S A
†F G Young	Cambridge, England

* Symposium secretary　　† Symposium chairman

CONTENTS

FOREWORD

By

J MAISIN

Chairman, Executive Committee, Council for International Organizations of Medical Sciences

It is a great pleasure for me to present this monograph on 'Experimental Diabetes and its Relation to the Clinical [illegible]

crine Interrelationships in Carbohydrate Metabolism' in London which preceded it and the Second International Congress of Biochemistry which followed, both attracted many world-renowned scientists and made it possible to group under the chairmanship of Professor F G Young a panel of distinguished physiologists, biochemists and clinicians

On behalf of the Council, I would like to thank all those who made the symposium a success the chairman, who guided the discussions with skill, good humour and clock-like precision, the members of the panel, the director of the Academisch Ziekenhuis at Leiden who graciously made available lecture hall and equipment, and finally Dr Gerritzen who acted as local organizer and made everything possible

I hope that the publication of the papers and of the discussions of the numerous aspects of experimental diabetes, many of which are still much debated, will be of value to all concerned with this branch of science

CHAIRMAN'S OPENING REMARKS

By

F G YOUNG

The reasons for the holding of a Symposium on 'Experimental Diabetes and its Relation to the Clinical Disease' at the present time are many and obvious, and we are indeed fortunate to be able to discuss this topic under the aegis of the Council for International Organizations of Medical Sciences in this ancient University of Leiden

The present time is particularly opportune since many of us have had the opportunity of debating recently at a Ciba Foundation Colloquium in London some of the many aspects of the hormonal control of carbohydrate metabolism which are relevant to our present Symposium, while others of us, not necessarily the same, have been able to consider diabetes in its widest aspect during the recent meeting here in Leiden of the International Diabetes Federation Who was responsible for the proposal that the C I O M S Symposium should be held in conjunction with the other two meetings I do not know for certain, but I suspect that Professor J P Hoet was the initiator of this excellent idea, and to him our grateful thanks are therefore due

In the present symposium our interest will be primarily devoted to diabetes in its experimental aspects, with especial reference to the genesis, treatment, and possible cure, of the clinical disease We shall undoubtedly spend much of the early part of our conference in considering the various methods which are now avail- [illegible]

shall probably have to touch upon albeit lightly, the more recondite topics of carbohydrate metabolism with which we were concerned during the early part of the Ciba Foundation Colloquium. Ultimately we shall consider some of the clinical observations which have been made in recent years, and here it is to be hoped we may succeed in providing at least a partial synthesis of the many lines of thought which will previously have run through our discussion

We are fortunate today in having before us the pre-circulated text of the contribution from each member of the Symposium, and in this connection I should like to express our thanks to Dr J F Delafresnaye and his staff, especially Mrs T K Tausig, for the excellent preliminary work they have undertaken on our behalf, and particularly for the excellent reproduction and rapid circulation of the texts now before us With the main communications already available we shall be able to spend most of our time, as it should be spent, in discussion As recorders we have Dr G H Smith and Dr L Brasseur, who will note our *discussion in English and in French respectively*, and in the published volume a full account of the discussion will constitute an essential part of the record of our activities Indeed discussion is the most significant reason for our presence here today

I would like to take this opportunity of expressing our thanks to Dr F Gerritzen and to Mr P Duis for undertaking the local arrangements which have made this meeting possible, and which have been carried out so expeditiously We are grateful also to the Director of the Academisch Ziekenhuis, who has granted us the use of this theatre

In conclusion, I am sure you would wish me to ask Dr Delafresnaye to convey to Professor J Maisin Chairman of the Executive Committee of C I O M S, our grateful thanks for being able to meet here this week for such a valuable purpose in such enjoyable surroundings

ON THE ISLETS OF LANGERHANS

By

CHARLES H BEST

Department of Physiology
and
The Banting and Best Department of Medical Research,
University of Toronto, Canada

INTRODUCTION

After a brief historical review I will select, for emphasis, certain aspects of this interesting subject In 1869, in the introduction to his article entitled *The Microscopic Anatomy of the Pancreas,* Paul Langerhans wrote 'There is indeed hardly another organ in which there is such glaring contrast between the brilliant results of physiological research and the complete darkness in the realm of anatomical knowledge' (20) The pancreas was known merely as a racemose gland in spite of the very early work of the Dutch investigator Regner de Graaf (8) and of Claude Bernard's demonstration that its secretion played a vital role in the digestion of carbohydrates, proteins and fats (2) Langerhans was a very young man, 22 years old, when he published this, the third of his scientific papers, and he was appropriately modest He wrote 'I can describe at most a few isolated observations which suggest a much more complicated structure of the pancreas than hitherto accepted' In introducing the newly recognized bodies, he stated 'The cell is a small irregularly polygonal structure The cells lie together, generally in considerable numbers, diffusely scattered in the parenchyma of the gland' [illegible]

[illegible]

role 'Dans le pancreas d'un homme adulte (supplicié) je retrouve ces îlots très nombreux et volumineux (je les désignerai provisoirement sous le nom d'îlots de Langerhans)'. Thus we read the thoughtful words which immortalized Langerhans They appear in one of a series of short articles by Laguesse in the *Memoires de la Société de Biologie*, 1893 (18)

In 1907, M A. Lane made an important advance in knowledge

of the islets (19). He developed definite procedures for the differentiation of the alpha- and beta-cells. The granules of the alpha-cells are relatively large and have large spherical nuclei with little chromatin. The granules are precipitated by 70 per cent alcohol The granules of the beta-cells are soluble in this concentration of alcohol but are precipitated by aqueous chrome-sublimate. They are much more numerous and considerably smaller than those of the alpha-cells and contain higher concentrations of chromatin

Professor R. R. Bensley, a graduate from Toronto in 1892 who has spent most of his active life in the U.S.A., has perhaps more than any other person of his generation informed us regarding [illegible]

demonstration of a system of fine anastomosing tubules about the pancreatic duct which *are in continuity* with the islets of [illegible]

clearly. Mr William Wilson (our senior histological technician), who made many of the preparations I am using, suggested that the publication of Professor Bensley's drawing and this photomicrograph in the same article would be of interest. I wrote to him recently to secure his reaction to the present situation. He answered promptly in his own firm hand 'I am afraid that my knowledge of recent developments is too meagre to give you much help. ... The record of the medical profession in the management of diabetes both pre-insulin and post-insulin has been a magnificent one and has my enthusiastic admiration. In the fundamental sciences, however, the tendency to a narrow point of view has been ever manifest. The preoccupation at the present time with the alloxan phenomenon is to my mind an evi- [illegible]

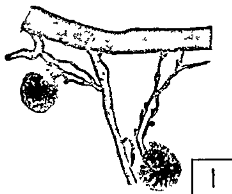


Fig. 1
I r t d t slet rel io isl p (gross)
Norr al guinea pig (× 70)
(Redrawn after B l y Maxi n w and Bloom *Textbo k f H l gy* th ed Fig 390)

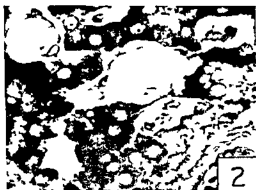


Fig.
I t reat f t t l t rel u l j (n r s j)
N rmal rat Wilson–Gomori method (800)



FIG 3
N r d g e t j c l t
Gon or chroi c haei atoxyli i phloxine n ethod (× 240)

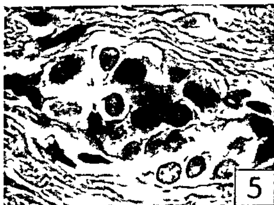


FIG 5
Dege erate l (d ct l g ted) d g pa icreas
H E ( )

as to promptness of autopsy and methods of preparation In that period the pathologist felt a sort of compulsion to adapt his findings to a popular theory and this compulsion spoilt his objectivity In those cases where reduction in number or size of the islets was not reported, obviously the pathologist was at his wit's end to explain the disease I think that it is important that workers in the fundamental sciences who are engaged in this field should be made to realize that, while plus or minus insulin is an important factor, the situation is far more complex The recent experiences with ACTH and cortisone help us to appreciate these facts, although I am sorry to say that the medical profession has not exercised the restraint and caution in the use of these hormones which was imposed in the case of insulin by the dramatic results of over- or under-dosage'

CONSIDERATIONS OF ISLET MORPHOLOGY AND FUNCTION

I will bring to your attention today some evidence supporting in a general way, Professor Bensley's view There are many facts, as he states which emphasize the importance of clinical diabetes As a physiologist, one could not accept normal appearance of a gland as a complete proof of normal function The histologist and the physiologist are not now, as in the past, commonly integrated in a single individual, but they can work together most profitably Using appropriate stains, my colleagues Drs Hartroft and Wrenshall find that the granule count in the cells may frequently run parallel to the amount of insulin extractable Indeed this had been observed in the earlier work on diet and insulin content of pancreas which my colleague Professor R E. Haist and I discussed some years ago Similarly in metadiabetes produced in dogs by purified growth hormone or by alloxan, the histologist can predict with reasonable accuracy what the insulin content will prove to be This is also true in 'growth-onset' diabetes in human subjects where the lesions in the cells are always advanced and the insulin content is consistently low But difficulties were encountered when the pancreases of 'maturity-onset' diabetics were studied and the correlation of granule count with insulin content was not nearly as satisfactory as in cases exemplifying other types of diabetes The basic cause of this situation is quite probably, that an extensive depletion of granules

becomes obvious and unmistakable, but when the decrease is slight, various other factors affect the picture and it is impossible for the histologist always to make an accurate interpretation

It is important and interesting to ask 'In how many diabetic patients is there no evidence of any abnormality in the manufacture or liberation of insulin?' Professor Bensley had referred to the fact that he was unable to detect any abnormalities in the islet cells in some diabetic patients at autopsy Schields Warren states that 26 per cent of the autopsies in his series of diabetics revealed essentially normal islets (24) Wrenshall, Bogoch and Ritchie found the average insulin content of the pancreas of maturity-onset diabetics to be approximately half that of non-diabetics (28) Based on units of insulin per gram of pancreas 6 8 per cent of the diabetics had values falling *above* the average of non-diabetics Based on units of insulin per square metre of [illegible] on units per gram of pancreas, or 21 per cent based on units per square metre of body surface Three diabetics out of fifty-nine had more total insulin in their pancreases than the average non-diabetic Twelve had values falling within the range of standard error for the non-diabetics

We are interested in the histological appearance of the islets and in their insulin content, but the deduction which we hope to be able to make from these studies is 'How much insulin in relation to the normal amount was this subject's pancreas providing for the tissues of the body?' The determination of the blood insulin level will give us an important part of the answer we seek, but this has yet to be placed on a quantitative basis Mr J M Salter, in our department, is just beginning to achieve satisfactory assays using the Gellhorn-Bornstein technique with some slight modifications and improvements I have been talking about blood insulin for a long time and I congratulate Dr Gellhorn, Dr Bornstein, Dr Groen and the others who have made the present methods available Bornstein and Lawrence have already provided us with some information on the level of blood insulin which can be maintained by the maturity-onset diabetics who have, on the average, about half the normal amount



Fig. 8

Gy [illegible] fi [illegible] [illegible] [illegible] [illegible] [illegible]

D g Cork [illegible] Sp [illegible] a [illegible] d abetes [illegible] sul [illegible] a [illegible] a ned 8 years Be t s Carmine sta

000

D (A Wr [illegible] hal)

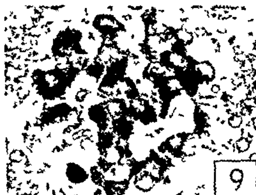


Fig. 9

[illegible] ell gran [illegible]

N [illegible] al [illegible] W [illegible] Co [illegible] aldeh de fu h [illegible] c h d

700

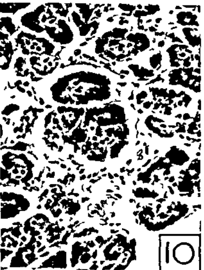


FIG 10

Small shrunken islet of Leonard Thompson (first insulin-treated human)

H/E (× 720)

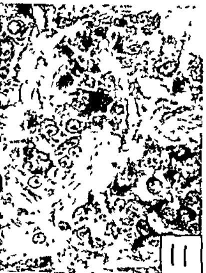


FIG 11

Normal appearing islet of Dr Joseph Gilchrist (first physician to receive insulin treatment)

Gomori's chrome haematoxylin phloxine method (× 700)



FIG 12

Beta-cell granulation in large islet in first recorded islet cell tumour

Wilson-Gomori aldehyde fuchsin (× 500)
(By courtesy of Wilder Allan and Kernohan)

of pancreatic insulin (4) This average figure may well prove to have little clinical significance since each case will have to be considered as an individual Some cases with high blood insulin may prove to require more exogenous insulin than others with a lower blood level As I have discussed elsewhere (3), we must also learn to assay the insulin antagonists in blood

I would now like to bring to your attention some other interesting pictures of islet cells In the normal guinea-pig's pancreas stained by Gomori's chrome alum-phloxine procedure (Fig 3) you will agree that there is no difficulty in distinguishing alpha- and beta-cells Similarly in the normal rat pancreas (Fig 4) the peripheral alpha-cells stand out clearly from the centrally located beta-type As originally shown by Arnozan and Vaillard (1884) (1) and by Schulze (1900) (22), ligation of the pancreatic ducts isolates the islets with waves of fibrous tissue (Fig 5) and it was from degenerated pancreas like this that Banting and I extracted our first insulin in 1921 Professor Lyman Duff, whom we are proud to claim as a Toronto graduate in Medicine (he is now Dean of Medicine at McGill) has kindly provided me with examples of what we used to call hydropic degeneration, but now know from the work of Duff and Toreson to be glycogen infiltration (6) (Fig 6) Similar conditions can be demonstrated in meta-hypophyseal diabetes in dogs where the glycogen is found in the degenerated islet and duct cells (Fig 7) These latter preparations were made available to me by Professor James Campbell, whose studies on the production of permanent diabetes in dogs by injection of highly purified growth hormone are now appearing in the press from our Department of Physiology In a case of spontaneous diabetes in a dog studied by Dr Wrenshall, glycogen infiltration of the duct cells was observed (Fig. 8) Dr Hartroft studied many sections of this pancreas but found no islets

HUMAN CASES OF SPECIAL HISTORICAL INTEREST

Turning now to human cases, I would like to record the fact that the first diabetic patient (L.T) treated with insulin, who died of broncho-pneumonia some eleven years after the day he received his first injection (11 January 1922), had an atrophied pancreas in which it was difficult to find any islet tissue The beta-cell granules stand out beautifully in the normal pancreas stained

by the Wilson-Gomori procedure (Fig 9) The small shrunken islet illustrated in Fig 10 is from Leonard Thompson's pancreas (I am indebted both to Dr Walter Campbell and to the Department of Pathology, University of Toronto, for permission to use this illustration) I have recorded elsewhere that it fell to my lot [illegible]

P

E

material to Drs Campbell and Fletcher for the first clinical trial The insulin was crude but active, and certain features of its preparation such as the concentration of ethanol which I adopted after preliminary trials and testings on diabetic dogs for the initial extraction of the pancreas, have been almost universally used for the past thirty years in the large scale production of insulin

I would like to sh [illegible]
the first doctor to
Dr Joseph Gilchrist
him his first injection of insulin in our laboratory in the winter of 1922 I followed his blood sugar downward and we collected [illegible]

content of the pancreas was 0 3 units per gram instead of the normal 2 4 units The total insulin content of the pancreas was 9 8 units instead of about 200 units The gland weighed 35 grams in contrast to a normal weight of 80 grams We can only guess at the output of insulin, but it was presumably a very small fraction of normal The figures were obtained by my colleagues Wrenshall and Hartroft from the specimen made available by Dr A J Blanchard of the Sunnybrook Military Hospital

The first case in which an excessive secretion of insulin was established by clinical and necropsy findings was that of Wilder, Allan, Power and Robertson (26) This patient, also a physician, had profound hypoglycaemia and an exploratory operation revealed a tumour mass in the pancreas with metastases in the liver Insulin was found in one of the liver metastases as well as in the

primary tumour Through the kindness of Dr Russell Wilder, Dr Frank Allan and Dr J W Kernohan I am able to show you sections from this historic case, stained in our laboratory by Hartroft and Wilson (Fig 12)

The first case of islet cell tumour to recover after surgical removal of the growth was that reported from Toronto by Howland, Campbell and Maltby (15) A tumour about 1 5 cm in diameter was removed by the late Roscoe Graham and although it was originally considered to be probably carcinomatous in nature, the pathologist, Dr W L Robinson, now considers that it may be classified as benign. A section from this insulin-containing tumour is shown in Fig 13

In the most recent collection of cases with islet cell tumours[1] three hundred and ninety-eight in number (14), there were three hundred and thirteen benign adenomas The carcinomas numbered thirty-seven and the others were questionably malignant In 12 6 per cent of cases there was more than one adenoma present

Various tissue experts have attempted to grow islet cell tumour tissue *in vitro* and to transplant the cells which had been acclimatized' in diabetic serum to diabetic patients Professor Gaillard in Leiden had a few encouraging results Dr Marjorie Murray at the Presbyterian Hospital in New York also secured good growth of cells, but on transference to patients there was no evidence of persistent growth of insulin-producing cells (25)

FACTORS AFFECTING ISLET VOLUME

Dr Haist and I made a series of studies on the effects of diet on the insulin content of the pancreas Fasting or feeding diets rich in fat greatly reduced the amount of insulin extractable Insulin administration lowered the insulin content but removal of the pituitary or adrenals had no effect (Fig 14) In meta-diabetes of all types the insulin content of the pancreas is greatly reduced or even nil

Since insulin is a product of the islets of Langerhans—and we believe that most cases of diabetes are characterized by either an absolute or a relative insulin deficiency—it is important to

[1] Dr Allan O Whipple s recent review *Islet Cell Tumours of the Pancreas* gives an excellent account of the historical and present aspects of this field (25)

learn more about the factors which control the total amount of islet tissue This problem has been approached by measuring the volume or weight of the islets of Langerhans under a variety of experimental conditions The weights have been estimated in the work directed by Haist by a special technique employing intravenous injection of neutral red, fresh tissue preparations and a planimetric method of measurement

Both dietary and hormonal factors have been found to influence islet growth If the intake of a balanced diet is so reduced that the individual fails to gain weight, the islets fail to grow (10, 11)

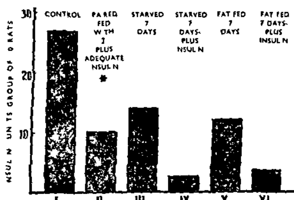


FIG 14
Effects of d et on insul n content of pancreas

effect of carbohydrate is to be found in the increase in islet tissue which results from the continuous infusion of glucose (10, 11, 27)

The hormonal factors influencing the islets are also numerous Insulin itself when given in very large amounts depresses the growth of the islets (7) The hormones which are apparently antagonistic to insulin tend to stimulate islet growth

In most instances where a trophic effect of the pituitary on a gland has been established, removal of the pituitary leads to its atrophy Hypophysectomy prevents the islets from growing



FIG. 4
Normal rat islet
Gomori chrome haematoxylin phloxine method
(× 1000)

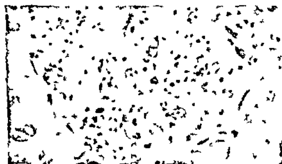


FIG. 6
Glycogen infiltration of islet cells
Male diabetic patient. Best's Carmine stain
(× 800)
(from ref. [illegible])

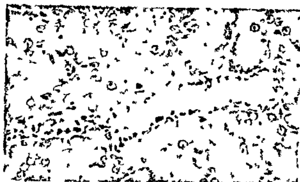


Fig 7
Glycogen fil ra of p ea c d ts
Gro vth Horn one diabetes B st s Car e sta n
(× 800)
(Dr James Can pl ell)



Fig 13
Hypert opl ed let cells i pr t si essf lly ope ated isl t t mo r
H/E (× 1160)
(Graham Ho vland Campbell and Maltby)

normally and thus may lead to some reduction in islet weight as compared with paired-fed control animals, but this difference is small when considered in relation to the decrease in size of adrenals or gonads after pituitary removal Hypophysectomy results in an atrophy of the acinar tissue of the pancreas (9 17) and thus increases the ratio of islet to acinar tissue in the hypophysectomized rats, even though the islet tissue *itself* has not increased in amount As Haist points out (10, 11) it illustrates one reason for not accepting a change in islet to acinar ratio *as an adequate sole criterion for a change in islet volume*

In intact rats the islets can be made to grow by the injection of crude saline extracts of the pituitary gland (10, 11 12), and in hypophysectomized rats also the injection of crude saline extracts or of purified growth hormone preparations leads to an increase in islet tissue (12) In both instances the islet growth is not greater than in paired-fed intact animals and may well be related to the food intake or body growth

The thyroid gland also influences the islets and probably the pituitary thyrotrophic material would do likewise The administration of desiccated thyroid for forty days or longer caused an increase in the weight of islet tissue as well as an increase in pancreas weight (10, 11) In one small series of hypophysectomized animals, thyroid administration was found by Haist and his colleagues to cause both islet and pancreas to increase in weight (12)

A considerable amount of work on the influence of the gonads on carbohydrate metabolism and the islets of Langerhans has been carried out by Ingle (16) and by Cardeza and Rodriguez (5) Cardeza (working in Professor B A Houssay's laboratory) reported an increase in the islet to acinar ratio in the pancreatic remnants following the administration of oestrogens to rats from which 95 per cent of the pancreas had previously been removed Dr Haist's group found that injection of stilboestrol in large doses led to increases in islet weight and in the ratio of islet weight to unit of body weight which were probably significant, though the increase in the ratio of islet weight to weight of the pancreas was not In the Toronto studies, progesterone in large doses also caused an increase in islet weight Removal of the gonads had no significant effect on islet growth A review of the effects of

sex hormones on experimental diabetes has recently been published by Houssay (13)

It is well established, of course, that the anterior pituitary is a very important factor in the growth of the animal as a whole Since the islet volume is so well correlated with body weight it may be that islet growth is related to the growth of the animal as a whole and to the effect which body growth has on insulin requirements Procedures which prevent body growth, such as restriction of caloric intake, also interfere with islet growth The manner in which the demand for insulin is transmitted from the cells of the body to those of the islets is not known It is possible that, directly or indirectly, an increase in blood insulin may set in motion physiological events which affect islet activity This is yet another set of obviously related physiological facts for which the mechanism of interrelation remains obscure Insulin may be the main anabolic hormone, and processes relating to fat, glycogen or protein formation may call for an increased supply

ACKNOWLEDGEMENTS

You will have perceived long before this that I am not an histologist Professor Hartroft and Mr Wilson have helped me to compensate for this deficiency by making special preparations of new and old material for me Dr Haist, a physician and physiologist like myself, has generously helped me summarize his recent advances My colleagues Dr James Campbell, a physiological chemist, and Dr Gerald Wrenshall, a physiological physicist, have assisted my presentation where it utilized findings obtained in their sections of our laboratories

REFERENCES

1 ARNOZAN,— and VAILLARD,— , *Arch Physiol norm Path*, 3me ser , 3, 287 (1884)
2 BERNARD, C , *Leçons de Physiologie Expérimentale*, Paris Baillière (1858)
3 BEST, C H , *Diabetes*, 1, 155 (1952)
4 BORNSTEIN, J and LAWRENCE, R D , *Brit med J*, ii 1541 (1951)
5 CARDEZA, A F and RODRIGUEZ, P R., *Rev Soc argent Biol*, 25, 178 (1949)
6 DUFF, G L and TORESON, W E , *Endocrinology*, 48, 298 (1951)
7 EVANS, M A and HAIST, R E , *Amer J Physiol*, 167, 176 (1951)

8 DE GRAAF, R., *Traité de la Nature et de l'Usage du Suc Pancréatique*, Leiden (1671), tr PACK, C, London N Brook (1676)
9 GRIFFITHS, M, *J Physiol*, 100, 104 (1941)
10 HAIST, R E, EVANS, M, KINASH, B, BRYANS, F E and ASHWORTH, M. A., *Proc Amer Diabetes Ass*, 9, 3 (1949)
11 HAIST, R E and KINASH, B, *Fed Proc*, 10, 58 (1951)
12 HAIST, R E *et al*, unpublished experiments
13 HOUSSAY, B A, *Brit med J*, ii, 505 (1951)
14 HOWARD, J M, MOSS, N H and THOADS, J E, *Surg Gynec Obstet*, 90, 417 (1950)
15 HOWLAND, G, CAMPBELL, W R, MALTBY, E J, and ROBINSON, W. L, *Trans Amer neurol Ass*, p 551 (1929), *J Amer med Ass*, 93, 674 (1929)
16 INGLE, D J, *Recent Progr Hormone Res*, 6, 159 (1951)
17 KOSTER, S, *Pflüg Arch ges Physiol*, 224, 212 (1930)
18 LAGUESSE, E, *C R Soc Biol*, *Paris*, 45, 819 (1893)
19 LANE, M A, *Amer J Anat*, 7, 409 (1907)
20 LANGERHANS, P, *Beitrage zur mikroskopischen Anatomie der Bauchspeicheldruse* (Dissertation), Berlin G Lange (1869)
21 RICHARDSON, K C and YOUNG, F G, *J Physiol*, 91, 352 (1937)
22 SCHULZE, W, *Arch mikr Anat*, 59, 491 (1900)
23 TEJNING, S, *Acta med scand*, 198, Suppl. (1947)
24 WARREN, S, *The Pathology of Diabetes Mellitus*, 2nd ed, Philadelphia Lea & Febiger (1938)
25 WHIPPLE, A O, *Canad med Ass J*, 66, 334 (1952)
26 WILDER, R M, ALLAN, F N, POWER, M H and ROBERTSON, H E, *J Amer med Ass*, 89, 348 (1927)
27 WOERNER C A, *Anat Rec*, 71, 33 (1938) 75 91 (1939)

DISCUSSION

YOUNG At the San Francisco meeting two years ago, Hartroft suggested that the liberation of insulin from the islets could be depressed or inhibited under certain conditions by the formation of fibrous tissue (HARTROFT, W S, *Proc Amer Diabetes* [illegible] 10, 1950) Are there any further comments on [illegible] idea?

BEST I think that some of Wrenshall's [illegible] do, [illegible] view Some more work has been [illegible]

Young Should we now speak of 'glycogen infiltration' rather than 'hydropic degeneration'?

Best *I prefer the term* 'glycogen infiltration' to 'hydropic degeneration'. I am wondering how much glycogen can be demonstrated chemically I suggest to Mrs Cori that the enzymic processes involved in this deposition of glycogen might be worth studying

Gerty Cori I have no comment to make on this But I should like to ask whether the glycogen level in the pancreas is dependent on a high blood sugar level

Best Duff has shown that glycogen is removed from the pancreas under the influence of small doses of insulin It is hard to answer this

Best Hartroft thinks that this is true hydropic degeneration and not glycogen infiltration

Lazarow What is the relation between the beta-cell granule and the amount of insulin stored in the cell? There appears to be a wide gap in our knowledge of the factors which influence the synthesis of insulin and the factors which control its storage and release from the cell

Best Valuable work on the solubility of the granules was done, as you know well by your mentor R R Bensley

Lazarow But why are the beta-cell granules insoluble in the cell? What controls the degranulation of the beta-cell and the release of insulin?

Best Counts of granules give a good correlation with insulin content if they are not too few in number We must try to correlate

glycogen deposition in the beta-cells should be studied Up to now, the latter has been regarded as a degenerative process called hydropic degeneration We now learn that, after recovery from a long period of induced diabetes, all of the beta-cells may be in a state of glycogen infiltration *and yet there is apparently normal insular function* Inasmuch as glycogen infiltration of the beta-cells is always associated with excessive functional demands upon them, it is possible that a pituitary factor which stimulates the beta-cells is responsible for this phenomenon I wonder whether induction of metahypophyseal

diabetes in the dog, followed by hypophysectomy plus adequate target-organ substitution therapy, would give rise to glycogen infiltration of the beta-cells In other words, spontaneous recovery from metahypophyseal diabetes would be a good place to study the influence of the pituitary gland upon this process

BEST No studies of this problem have been made in my laboratories

LONG Neural control of insulin secretion has been studied by [illegible] find no change The evidence suggests that if there is any neural control it must play a very minor part

YOUNG Houssay did get a very small effect

LUKENS I should like to ask Professor Best whether any of the staining methods in use in his laboratory for the determination of the insulin content of the pancreas are applicable to post-mortem material

BEST Methods for the study of post-mortem material are given in Wrenshall's publications It is amazing how much insulin remains in post-mortem material. If enough non-diabetic controls are run the experiments can be conducted very satisfactorily Wrenshall finds that there is no change in the insulin content of strips of pancreas stored at 4°C for periods of up to one month

VERZÁR Professor Best mentioned that the islets of the duck contain only alpha-cells Is there any insulin or only glucagon in the duck's pancreas?

BEST Wrenshall and Vuylsteke found the amount of insulin extractable from the duck's pancreas to be very low I suggest that it might therefore be a very good source of glucagon (See footnote to Professor de Duve's paper—*Ed*)

LUKENS Is there any form of experimental diabetes in which hyaline change is the predominant lesion There is a paper by Bensley in the press which shows that the amount of hyaline change is not correlated with the severity of the diabetes I think—though it is a completely unsupported theory—that the hyaline change is a result of inflammatory lesions

BEST This question has been discussed before

YOUNG I am interested by Dr Lukens' statement on hyaline change Is this a general correlation?

LUKENS Most pituitary diabetics, and also alloxan diabetics, show atrophy of the islets

YOUNG Richardson and I found that there was complete hyalination of the islets in our dogs with metahypophyseal diabetes. There

seems to be a very great difference between the dog and the cat in this respect

LUKENS We worked with both dogs and cats but did not find hyalination in either

which could be seen, even in the neuro-insular complex, nevertheless the animal recovered from the diabetes without treatment In this
which lasted
le that when
iged start to
secrete sufficient insulin? We had no facilities for estimating the pancreatic insulin in these animals and, because they are very lengthy, few experiments were done

BEST We have had no experience on this point, but I would expect to find some granules

YOUNG Richardson was, I think, fairly sure there were no stainable granules in the beta-cells

BEST We should perhaps look into this again with new stains I suppose, however, that you might well have quite a lot of insulin and very few granules

LAZAROW We found a similar spontaneous disappearance of the diabetes in rats which had had alloxan diabetes for periods of one year or more The islets of these animals did not show hydropic degeneration Although islet regeneration does not appear to be a prominent

diabetes? Also is there a reduction in the amount of acinar tissue in such cases?

BEST Several of the first cases of human diabetes treated with insulin had very small pancreases

LONG It may be that the type with the small pancreas is associated with one of Dr Lawrence's classes of diabetes, while that with a normal sized pancreas may fall into the other group

LAWRENCE In my experience very long standing diabetics have very small pancreases but no histological staining has been done

LONG The whole pancreas is reduced in size in these cases, not just the islets alone

control, one is incubated with an excess of purified glucagon and three are incubated with intermediate doses of this substance The data obtained are used to construct the sensitivity curve of the particular liver The three remaining slices are incubated with unknowns The results are read from the sensitivity curve and are expressed directly in micrograms of the standard glucagon preparation

SITE OF FORMATION AND HORMONAL NATURE

It has been shown by Sutherland and de Duve (28) that glucagon is produced by the pancreatic islets and that this process is not affected by alloxan The conclusion was drawn that the alloxan resistant alpha-cells of the islets are probably the main site of formation of glucagon although in some species the [illegible] the gastric mucosa and

[illegible] direct demonstration [illegible]

selective injuries to the alpha-cells of the pancreatic islets The glucagon content of the pancreas has been found to be reduced by approximately sixty per cent as a result of this treatment (40) Animals poisoned with cobalt lose a considerable amount of weight, but it is unlikely that this factor is responsible for the observed decrease, since neither the glucagon level nor the alpha-cells of the pancreas were significantly influenced in guinea-pigs similarly reduced by starvation (41) Small amounts of cobalt inhibit the glycogenolytic effect of pure glucagon on isolated liver slices, but the extracts of cobalt-treated pancreas were not,

[illegible]

into rabbits The subsequent hypoglycaemia was of normal magnitude [2]

[1] Following the observation mentioned by Professor Best at this symposium that the [illegible] contain a very large proportion of alpha-cells, the glucagon content [illegible] has been assayed in our laboratory The pancreas of ducks has been [illegible] es the amount of glucagon present in mammalian pancreas.

PURIFICATION AND ASSAY

The samples of glucagon used in our experiments have been prepared from alkali-inactivated insulin according to the method described by Sutherland *et al* (29) The results obtained parallel those of these authors and the purity of the various samples isolated ranged between 80 and 100 per cent of that of their purest preparation It is, however, doubtful whether it is 'native glucagon' that is thus prepared, since it has been shown that alkali treatment causes some degradation of this substance (27)

The assay of glucagon is based on its ability to increase the glucose output of isolated rabbit liver slices As shown by Sutherland and de Duve (28), the excess glucose produced above the output of control slices increases regularly with increasing dosage of glucagon, up to a certain limit which characterizes the maximal activity The absolute amounts produced vary considerably from one experiment to another, but the results become comparable if they are expressed as a percentage of maximal activity According to Sutherland *et al* (29) the potency of [illegible]
(or [illegible]
to [illegible]
standardized conditions

This statement implies that all livers react alike and that there are no individual variations in the glucagon sensitivity of the tissue Our own experience of the method, based on more than one hundred assays does not support this assumption (40, 42) For example, in six out of seven consecutive assays of a purified sample of glucagon, half-maximal stimulation was obtained with doses varying between 0 5 and 1 1 micrograms (as compared with 0 7 micrograms for Sutherland's purest preparation (29)) and in the seventh with as much as 4 5 micrograms The reliability of the last value was confirmed by the low reactivity of the liver to two other samples included in the test It should be mentioned that the rabbits used were purchased weekly from the local market and kept in the laboratory on a high carbohydrate diet for at least seven days before being used

In view of the variability encountered, the method has been modified as follows routinely, the test is made in triplicate on three sets of eight matched slices, and of these one serves as

adrenaline. In another group the excretion of urea was followed during the experiment. It decreased slightly with glucagon and remained practically unaltered with adrenaline. Glucagon alone caused hyperglycaemia, a considerable reduction of the liver glycogen level, no detectable change in the muscle glycogen level and an increased excretion of urea.

From these experiments it would appear that glucagon can act as a complete insulin antagonist. It is particularly noteworthy that the carbohydrate stores of the organism were not depleted as the result of the combined action of the two agents. If the antagonistic action of glucagon were due solely to its glycogenolytic effect, one would expect the hepatic glycogen content, or at least the sum of hepatic and muscular glycogen contents, to be decreased in proportion to the excess glucose utilized by other pathways. Such is indeed the case in the experiments with adrenaline, where the decrease of muscle glycogen is sufficient to account for the extra consumption of glucose. But this decrease does not occur in the experiments with glucagon, and one is led to suspect either that glucagon promotes gluconeogenesis—a hypothesis which appears to be disproved by the results on urea excretion—or that it inhibited the other effects of insulin. Since fed animals were used, there remains the possibility that glucagon enhanced the rate of absorption of glucose through the gut.

It is too early to assess the significance of these data. Let it simply be mentioned that the following facts may or may not be relevant to their understanding.

(i) The inhibition by pancreatic extracts of the effect of small doses of insulin on the glucose consumption of the isolated rat diaphragm (7).

(ii) The delay taken by the rat diaphragm in building up insulin resistance after injection of growth hormone (13).

(iii) The increased secretion of a substance resembling glucagon by the pancreas of animals treated with growth hormone (2).

(iv) The stimulation of the adrenal cortex by glucagon (17).

GLUCAGON AND ADRENALINE

Glucagon resembles adrenaline closely in its ability to stimulate glycogenolysis in liver tissue. It seemed that [illegible]

However, the problems of glucagon and insulin still remain intimately linked Much of the evidence in favour of the hormonal nature of the hyperglycaemic factor is derived from experiments indicating that the alpha-cells of the islets secrete a hormone which antagonizes the hypoglycaemic action of insulin (see above) On the other hand, the little evidence there is concerning the circumstances under which glucagon is secreted suggests that hyperglycaemia rather than hypoglycaemia is one of the stimuli for its secretion (4, 16) If this is true, glucagon would turn out to be a physiological synergist of insulin, at least under some conditions, and many of the results obtained in early work with mixtures of glucagon and insulin may still be relevant to the understanding of their joint action Finally, the results already mentioned of Bornstein *et al* (2) raise the possibility that glucagon may be involved in the pathogeny of pituitary diabetes

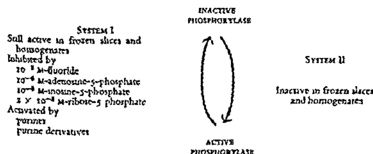
In view of these various facts, it appeared of interest to study the metabolic consequences of the administration of balanced mixtures of the two agents Early work had shown that the constant infusion of glucagon-containing insulin to rabbits pretreated with large amounts of uncontaminated insulin leads to a corresponding decrease of the amount of glucose required to keep these animals at a normal blood sugar level If sufficient amounts of the contaminated material are given, no glucose at all is required (14) Similar results have been obtained by administering adrenaline to insulinized rabbits (13, 23)

Recently Tyberghein has repeated these experiments, using an improved infusion technique and purified insulin-free glucagon (35, 36) Fed rabbits receiving 30 units of insulin (Novo) intravenously at the onset of the experiment and every 90 minutes subsequently, were kept at an approximately normal blood sugar level for as long as six hours by the constant intravenous infusion of glucagon, at the rate of 0 31 mg per hour, and of adrenaline at the rate of 0 17 mg per hour At the end of the experiment the hepatic glycogen level was not significantly different, in either of the two groups, from the value found in control animals receiving no insulin and injected with equivalent amounts of saline There was no significant change in the muscle glycogen level in the experiments with glucagon, whereas this level was reduced considerably in the experiments with

As will be seen, the main differences between the two substances concern their chemical properties (I a, b, c) site of formation (II) and effects on the cardio-vascular system (III f, VI a, b) In addition, glucagon does not appear to share the ability of adrenaline to stimulate glycogenolysis in muscle (III c, d, V c) The data on the activity of subcutaneously administered glucagon (IV) have been included because there have been claims that it is inactive when injected by this route (24, 25, 44) The lack of activity observed by other authors is explained by the presence of active insulin in their samples

Glucagon shares the ability of adrenaline to stimulate the adrenal cortex, as evidenced by the fall in the eosinophil count (III g) Whether similar mechanisms are involved with the two substances is not known (see above) The results of compensation experiments with insulin have already been discussed (V a b, c, d)

A problem of considerable interest is that of the mechanism of action of the two glycogenolytic agents (VII c, IX b) In a series of important investigations Sutherland and Cori (27, 30 31, 32, 33) have established that glucagon, as well as adrenaline, increases the amount of active phosphorylase in the liver by shifting the balance between two highly active antagonistic systems which regulate this activity Fig 1 summarizes their findings



Effect of glycogenolytic agents increase of rate of reaction II relative to that of reaction I inactive on system I after disruption of cell structure

FIG 1

Properties of the phosphorylase system
(from data of Sutherland and Cori 31

determine the extent to which this similarity holds true in other respects Table I summarizes the available data (References to classical work have not been included in this table)

TABLE I

Comparison between adrenaline and glucagon

Property	*Adrenaline*	*Glucagon*	*References*
I *Chemistry*			
(a) Dialysis	Dialysable	Undialysable	3 27 28
(b) Action of 0 1 N KOH 3 hr 38°C	Destroyed	Not destroyed	
(c) Action of proteolytic enzymes	Not destroyed	Destroyed	
II *Site of formation*	Adrenal medulla	Alpha-cells of pancreatic islets (Digestive mucosa)	28 40
III *Effect of intravenous injection*			
(a) Blood sugar	Increased	Increased	
(b) Liver glycogen	Decreased	Decreased	35 36
(c) Blood lactic acid	Increased	Unchanged	30 39
(d) Muscle glycogen	Decreased	Unchanged	35 36
(e) Urea excretion (rabbit)	Increased	Increased	36
(f) Blood pressure (rabbit)	Increased	Unchanged	25 39
(g) Eosinophil count (dog)	Decreased	Decreased	39
IV *Effect of subcutaneous injection*			
Blood sugar	Increased	Increased	39
V *Effect of continuous intravenous injection for 6 hours in insulinized rabbits*			35 36
(a) Blood sugar	Unchanged	Unchanged	
(b) Liver glycogen	Unchanged	Unchanged	
(c) Muscle glycogen	Decreased	Unchanged	
(d) Urea excretion	Unchanged	Slightly decreased	
VI *Effect on isolated frog heart*			39
(a) Amplitude of contraction	Increased	Unchanged	
(b) Frequency	Increased	Unchanged	
VII *Effect on isolated liver slices*			
(a) Glucose output	Increased	Increased	27
(b) Glycogen	Decreased	Decreased	27
(c) Phosphorylase activity	Increased	Increased	31, 32 33
VIII *Effect on homogenized frozen liver slices*	No action	No action	
IX *Effect on isolated rat diaphragm*			
(a) Glycogen	Decreased	?	43
(b) Phosphorylase activity	Increased	?	32 33
(c) Glucose consumption	Decreased	?	43
(d) Glucose consumption + insulin	?	*Decreased*	7

Despite a great number of different experimental approaches, including studies of the concentration and intracellular distribution of inorganic phosphate under various conditions, experiments with added hexosephosphates, investigations of the accessibility and degree of branching of glycogen etc, not the slightest indication could be obtained that the glycogenolytic agents modify the equilibrium conditions of the phosphorylase reactions, at least in isolated liver slices

In a second series of experiments the accessibility of the reactivated phosphorylase to added glucose-1-phosphate was explored It was found that, contrary to the behaviour of fresh slices or of preincubated slices subsequently enriched in phosphorylase by addition of fluoride, slices reactivated with glucagon *do not as a rule liberate more inorganic phosphate from glucose-1-phosphate* at pH 6 in a test of short duration, than do control slices which have not been similarly reactivated In other words, they behave as though the extra phosphorylase which is built up in them under the influence of glucagon and which can be demonstrated in a similar test performed on an homogenized slice, were not freely accessible to glucose-1-phosphate However, too many factors are involved in experiments of this type and attempts to account for every one of them in an unambiguous manner become rapidly defeated by the technical difficulties involved Several other interpretations can be offered for the observed differences, and the one mentioned has only the value of giving a slight indication.

In concluding, the possibility must be mentioned that our search was practically fruitless because there was nothing to search for Liver slices do not show all the properties of the intact organ *in situ* and may not have kept the ability to react irreversibly to a glycogenolytic agent For instance, no action of insulin has yet been clearly demonstrated on isolated liver slices If the irreversibility factor is associated with an inhibition of the action of insulin on the uptake of glucose by the liver, it will of course escape detection in the same system. An effect on the local phosphate concentration might similarly fail to show up in a system which, according to our investigations shows distinct signs of an impaired phosphate metabolism

When liver slices are incubated *in vitro*, the balance is in favour of system I and the phosphorylase level falls If a glycogenolytic

that identical results can be obtained by adding 0 1 M-fluoride, suggesting that the two systems are continuously active and that system II can be favoured simply by inhibiting system I However, the latter is not inhibited by the glycogenolytic agents in soluble systems, and the specific effect of these agents, like the ability to reactivate phosphorylase, is abolished by disruption of the cell structure For this reason Sutherland believes that the glycogenolytic agents are activators of system II The existence of a similar adrenaline-sensitive system in muscle has recently been reported by Sutherland (32, 33) He does not mention having tried glucagon on muscle

The mechanism of action of glucagon has also been investigated in this laboratory (42) When Sutherland's data became known, various experiments were directed towards the study of the phosphorylase system His results have been fully confirmed

still occurred to a limited extent in nitrogen, it was greater in air and still greater in oxygen Dinitrophenol (5×10^{-5} M) causes a significant reactivation of phosphorylase when added alone, and this effect is superposed on that shown by fluoride when the two substances are added together It appears, therefore, to behave as a true activator of system II On the other hand, the effects of glucagon or adrenaline and of fluoride are not additive It is therefore possible that they act by stimulating the release of a natural inhibitor of system I, rather than by activating system II

However, our main investigations have been concerned with a search after the 'irreversibility factor' in the action of the glycogenolytic agents Obviously, the increase in phosphorylase activity does not explain how these agents *inhibit* the synthesis of liver glycogen, for instance, in animals loaded with glucose A second factor must therefore be involved in their action

19 LUKENS, F. D. W., *J. clin. Endocrin*, 12, 246 (1952).
20 LUNDSGAARD, E, NIELSEN, N A and ORSKOV, S. L, *Skand Arch. Physiol*, 81, 11 (1939)
21. MALANDRA, B and CAVALLERO, C, *Rass Fisiopat clin terap*, 22, 1 (1950)
22 MIRSKY, A, FUTTERMAN, P, WACHMAN, J and PERISUTTI, G, *Endocrinology*, 49, 73 (1951)
23 DE NAYER, P P, *C R Soc Biol*, *Paris*, 111, 1049 (1932)
24 OLSEN, N S and KLEIN, J R, *Proc Soc exp Biol*, *N Y*, 66, 86 (1947)
25 PINCUS, I J, *J clin Endocrin*, 10, 556 (1950)
26. SCOTT, D A and FISHER, A M, *Biochem J*, 29, 1048 (1935)
27 SUTHERLAND, E W and CORI, C F, *J biol Chem*, 172, 737 (1948)
28 SUTHERLAND, E W and DE DUVE, C, *J biol Chem*, 175, 663 (1948)
29 SUTHERLAND, E W, CORI, C F HAYNES, R and OLSEN, N S, *J biol Chem*, 180, 825 (1949)
30 SUTHERLAND, E W, *Recent Progr Hormone Res*, 5, 441 (1950)
31. SUTHERLAND, E W and CORI, C F, *J biol Chem*, 188, 531 (1951)
32 SUTHERLAND, E W, in *Phosphorus Metabolism*, vol 1, ed McELROY, W D and GLASS B, p 53, Baltimore The Johns Hopkins Press (1951)
33 SUTHERLAND, E W, *Ann N Y Acad Sci*, 54 693 (1951)
34 THOROGOOD, E and ZIMMERMANN B *Endocrinology*, 37, 191 (1945)
35 TYBERGHEIN, J, *Arch int Physiol*, 60, 105 (1952)
36 TYBERGHEIN, J, unpublished experiments
37 VAN CAMPENHOUT, E and CORNELIS, G *C R Soc Biol*, *Paris*, 145, 933 (1951)
38 VAN CAMPENHOUT, E and CORNELIS, G, *Bull Acad Méd Belg*, 16, 382 (1951)
39 VUYLSTEKE, C A, DE DUVE C and NYS, A, *Arch int Physiol*, 57, 445 (1950)
40 VUYLSTEKE, C A, CORNELIS, G and DE DUVE, C, *Arch int Physiol*, 60, 105 (1952)
41 VUYLSTEKE, C A, CORNELIS, G and DE DUVE, C, unpublished experiments
42 VUYLSTEKE, C A and DE DUVE, C, unpublished experiments
43 WALAAS, O and WALAAS E, *J biol Chem*, 187, 769 (1950)
44 WEISBERG, H F, CAREN, R, HUDDLESTUN, B and LEVINE, *Amer. J Physiol*, 159, 98 (1949)

CONCLUSION

It will be clear from the preceding account that the data available today on glucagon raise more questions than they can answer Its hormonal nature appears to be fairly well established, and there are various hints that it may one day be fitted without too much difficulty into our schemes of metabolic regulations and perhaps help to clarify some of the problems which are puzzling us today To speculate beyond this would be unwise at the present juncture and the results of further work must be awaited

REFERENCES

1 ABEL, J J, GEILING, E M K, ROUILLER, C A, BELL, F K and WINTERSTEINER, O, *J Pharmacol*, 31, 65 (1927)
2 BORNSTEIN, J, REID, E and YOUNG, F G, *Nature, Lond*, 168, 903 (1951)
3 BURGER, M and BRANDT, W, *Z ges exp Med*, 96, 375 (1935)
4 [illegible]
5 [illegible]
6 [illegible] 89 (1947)
7 CANDELA, J L R -, TAMARIT, J, CANDELA R R - and CRISTENSEN-KONIG, W, *Arch Med exp*, 14, 11 (1951)
8 CANDELA, J L R -, *J clin Endocrin*, 12, 245 (1952)
9 CAVALLERO, C and MALANDRA, B, *Boll Soc ital Biol sper*, 25, 1 (1949)
10 CAVALLERO, C and MALANDRA, B, *Fol endocrin*, *Pisa*, 3, 405 (1950)
11 CAVALLERO, C and MALANDRA, B, *Arch Sci biol*, *Ital*, 34, 138 (1950)
12 DRAGSTEDT, L R, ALLEN, J G and SMITH, E M, *Proc Soc exp Biol*, *N Y*, 54, 292 (1943)
13 DE DUVE, C, MARIN, G and BOUCKAERT, J P, *Arch int Pharmacodyn*, 72, 29 (1946)
14 DE DUVE, C, HERS, H G and BOUCKAERT, J P, *Arch int Pharmacodyn*, 72, 45 (1946)
15 DE DUVE, C, *Rev med Liege*, 6, 258 (1951)
16 FOA, P P, WEINSTEIN, H R and SMITH, J A, *Amer J Physiol*, 157, 197 (1949)
17 GEILING, E M K and DE LAWDER, A M, *J Pharmacol*, 39, 369 (1930)
18 KRAHL, M E, *Ann N Y Acad Sci*, 54, 649 (1951)

ACTION OF THE HYPERGLYCAEMIC FACTOR (GLUCAGON) OF THE PANCREAS

By

J. L. R.-CANDELA

Instituto de Medicina Experimental, Madrid, Spain

INTRODUCTION

No sooner had insulin been discovered by Banting and Best than Macleod (1922) demonstrated that the insulin preparations used until then also had a hyperglycaemic effect. In 1923, Murlin *et al.* observed the so-called diphasic action of pancreatic extracts on the blood sugar level (17). Murlin therefore postulated that two substances are secreted by the pancreas: insulin, which has a hypoglycaemic action, and another, glucagon, which causes the blood sugar level to rise. A short time later, Bürger (1930), Burger and Kramer, and others remarked on the same fact and admitted the possibility of there being two pancreatic hormones (3, 6).

Some time elapsed before attention was given to elucidating the mechanism of this hyperglycaemic effect, which can be observed with the great majority of insulin preparations when injected intravenously. Then, a few years ago, great interest arose over this problem and there are now a large number of published papers on this subject. Perhaps two events have contributed to this increase in interest—a clinical one and an experimental one—and both took place quite recently. On the one hand there are the numerous observations of patients who have suffered a total pancreatectomy; after the operation the insulin requirement (40–50 units per day) is less than that necessary to maintain the blood sugar level of diabetic patients within the normal limits (14, 23, 24). Goldner and Clark (15) and others have also pointed out that the sensitivity to insulin increases after the removal of the pancreas. On the other hand there is the discovery that various forms of experimental diabetes require less insulin for their control after the removal of the pancreas.

Because of their interest, two questions [illegible] hyperglycaemic-gl[illegible] disc[illegible]

45 YOUNG, F G, *Schweiz med Wschr*, 76, 894 (1946)
46 ZIMMERMANN, B and DONOVAN, T J, *Amer J Physiol*, 153, 197 (1948)
47 ZIMMERMANN B, *Endocrine Functions of the Pancreas*, Springfield, Ill C C Thomas (1952)

DISCUSSION

see p 44

of fifty per cent was noticed, and in none of them were signs of malnutrition or loss of weight noticeable

Bornstein, Reid and Young have demonstrated that under the influence of growth hormone, the islets of Langerhans liberate the hyperglycaemic factor (1)

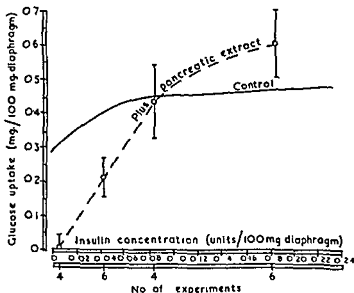


Fig 1

Glucose upt ke by the isolated rat d aphragm in the presence of insul n influence of an extract of a normal dog s pancreas

MECHANISM OF ACTION OF GLUCAGON

In 1929, Burger and Kramer published their first account of the effect of glucagon on the hepatic glycogen store and the subsequent rise in the blood sugar level (5) Some time later, Burger and Klotzbücher demonstrated the existence of a substance which appears in the plasma after the blood sugar tolerance test has been performed and which they identified as glucagon (4)

Shipley and Hümel showed in 1945 that more sugar was liberated by rat liver . . . um in the presence than in the absence of . . . rt and de Duve have

tions originally present in the pancreas? and (ii) What is its probable mechanism of action?

PRESENCE OF GLUCAGON IN THE PANCREAS

Clinical and experimental observations support the theory that the hyperglycaemic factor is in the pancreas originally, but there is no definite proof of it Dixon, Comfort and Lichman and Ricketts, Brunchswig and Knowlton have published two cases of total pancreatectomy following cancer in the pancreas of diabetic patients (12, 19) In neither of the two cases did the insulin requirement increase after the pancreatectomy, in one of them, in fact, it decreased No signs of poor nutrition were observed, although apparently the small insulin requirement should have been accompanied by more evident clinical evidence of malnutrition, if only as a result of disturbances of absorption On the other hand, Pincus treated a small number of diabetic patients with H-G factor and found that in five out of six patients with labile diabetes a greater increase in the blood sugar level was produced than that observed in normal persons (18)

Never has a patient been seen in a clinic with a tumour in the alpha-cells, nor has one been found at autopsy A tumour of normal appearance has apparently been found in the pancreas in a case of hypoglycaemia, though in this case the islets of Langerhans were lacking in alpha-cells (aplasia), owing to an anomaly of development (16)

From an experimental point of view, Young has proved that *in pituitary diabetes the removal of the pancreas is followed by* a reduction in the requirement for insulin and an increased sensitivity to the hormone (25) Dragstedt *et al* observed similar effects using animals with sub-total pancreatectomy (13) and Thorogood and Zimmermann in animals with alloxan diabetes (22) Candela *et al* have studied the insulin requirement of dogs with alloxan diabetes, first after ligation of the pancreatic ducts and finally after performing total pancreatectomy (7, 10) The results show that there is no change in the insulin requirement after ligaturing the pancreatic duct, even after a few weeks have elapsed and the exocrine tissues have atrophied On the other hand the requirement for insulin diminishes immediately after the performance of a total pancreatectomy In one case a fall

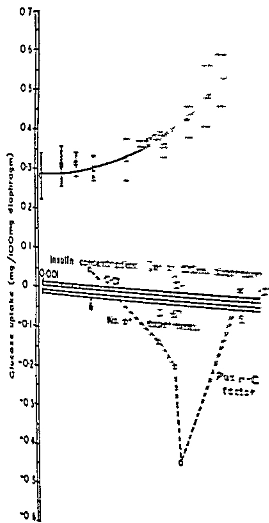


Fig. 1

Glucose uptake by the isolated rat diaphragm

pointed out that Danish insulin (Novo) does not produce such effects (2) Sutherland and Cori have carried out interesting work on the effect *in vitro* of insulin containing the hyperglycaemic factor (21) They used, as test, liver slices under aerobic or anaerobic conditions From these experiments one gathers that the glycogenolytic effect of insulin plus the H-G factor is on the phosphorylase system and depends on the integrity of the cellular structure Such an effect is specific since no glycogenolytic effect has been found using other proteins

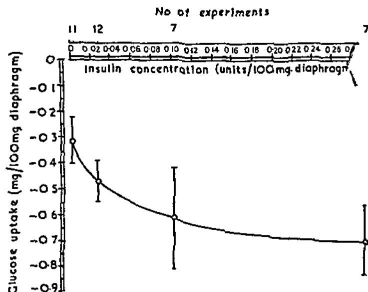


FIG 2

Glucose uptake by the isolated rat diaphragm in the presence of insulin influence of an extract of a alloxan diabetic dogs pancreas

We have studied the effect of pancreatic extracts of normal and alloxan diabetic dogs on the glucose uptake by insulin in the isolated diaphragm of a rat (9 11) from the pancreas of a normal dog has an inhibitory the glucose uptake, especially if the amount of insulin added small (Fig 1) The effect is greater if the pancreatic extract is made from a dog with alloxan diabetes (Fig 2) In this case the glucose uptake is negative, that is, less glucose is taken up by the

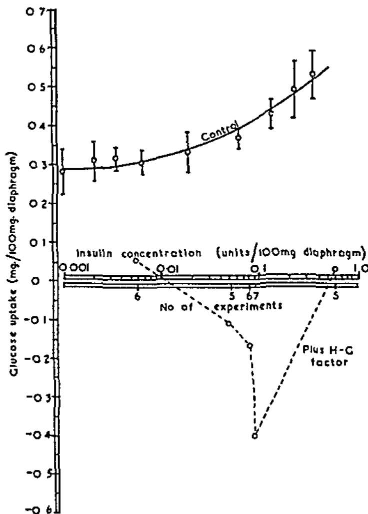


Fig 3
Glucose uptake by the isolated rat diaphragm in the presence of insul n influence of the hyperglycaem c factor

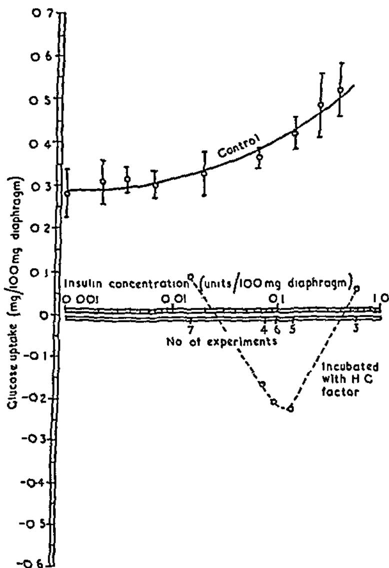


FIG 4
Gl icose uptake by the isolated rat d aphragm in the presen e of insi l n i ifluen e of previo is incubat on with the hyperglycaem c factor

diaphragm in the presence of insulin plus glucagon than in the absence of insulin

We have also investigated the action of the purified hyperglycaemic factor on glucose uptake (Fig 3) and the effect of previous incubation with the factor on the glucose uptake subsequently induced by insulin (Fig 4) (8) The results obtained are *all similar to those of the experiments with pancreatic extracts* This leads us to believe that, besides the action on hepatic phosphorylase, which results in hyperglycaemia, there may be another *mode of action near the muscle, which amongst other effects,* could be an act of competition

ACKNOWLEDGEMENTS

We wish to thank Dr Kirtley of the Eli Lilly Research Laboratories who so kindly provided the sample of hyperglycaemic factor used in our work. We also wish to thank the Ibys Institute for the help which they have given

REFERENCES

1 BORNSTEIN, J, REID, E and YOUNG, F G *Nature, Lond*, 168, 903 (1951)
2 BOUCKAERT, J P and DE DUVE, C *Physiol Rev*, 27, 39 (1947)
3 BÜRGER, M, *Klin Wschr*, 9, 104 (1930)
4 BÜRGER M and KLOTZBUCHER, E *Z ges inn Med*, 2 43 (1947)
5 BÜRGER, M and KRAMER, H, *Z ges exp Med* 67, 441 (1929)
6 BÜRGER, M and KRAMER H, *Arch exp Path Pharmak*, 156, 1 (1930)
7 CANDELA, J L R-, *An Acad méd-quir*, 29, 283 (1945)
8 CANDELA, J L R-, and CANDELA, R R-, unpublished experiments
9 CANDELA, J L R-, TAMARIT, J, CANDELA, R R- and CRISTENSEN-KONIG, W, *Arch Med exp*, 14 11 (1951)
10 CANDELA, J L R-, GOMI P M, CALDERO, M R and GONZALEZ-CARRERAS, P, *Trib Inst nac Cienc méd*, Madr, 9 89 (1947)
11 CANDELA, J L R-, TAMARIT, J, CANDELA R R- and CRISTENSEN-KONIG, W, *Arch Med exp*, 14, 91 (1951)
12 *DIXON, C F, COMFORT, M W and LICHTMAN, A L, Arch Surg, Chicago*, 52, 619 (1946)

13 DRAGSTEDT, L. R, ALLEN, J G and SMITH, E. M, *Proc Soc exp Biol, N Y*, 54, 292 (1943)
14 GASTON, E A, *New Engl J Med*, 238, 345 (1948)
15 GOLDNER, M G and CLARK, D E, *J Clin Endocrin*, 4, 194 (1944)
16 MCQUARRIE, I, BELL, E T, ZIMMERMANN, B and WRIGHT, W S, *Fed Proc*, 9, 337 (1950)
17 MURLIN, J R, CLOUGH, M D, GIBBS, C B F and STOKES, A M, *J biol Chem*, 56, 253 (1923)
18 PINCUS, I J, *J clin Endocrin*, 10, 556 (1950)
19 RICKETTS, H T, BRUNSCHWIG, A and KNOWLTON, K, *Amer J Med*, 1, 229 (1946)
20 SHIPLEY, R A and HUMEL, E J, *Amer J Physiol*, 144 51 (1945)
21 SUTHERLAND, E W and CORI, C F, *J biol. Chem*, 172, 737 (1948)
22 THOROGOOD, E and ZIMMERMANN, B, *Endocrinology*, 37, 191 (1945)
23 WAUGH, J M, DIXON, C F, CLAGETT, O T, BOLLMAN, J L, SPRAGUE, R G and Comfort M W, *Proc Mayo Clin*, 21, 25 (1946)
24 WHIPPLE A O, *Ann Surg*, 124 991 (1946)
25 YOUNG, F G, *New Engl J Med*, 221, 635 (1939)

DISCUSSION OF PAPERS BY PROFESSORS DE DUVE AND CANDELA

YOUNG *It would be valuable if we could agree on terminology* and, like Professor de Duve, now adopt the name 'glucagon'

DE DUVE The substance was first called 'the hyperglycaemic-glycogenolytic factor' by Sutherland and myself as we did not think *that 'glucagon' was a very apt name* I am still of that opinion but now feel that we should respect the wishes of Burger who has done the pioneer work in this field In any case, 'hyperglycaemic-glyco-
[illegible] *for the name of a hormone*

[illegible] had an answer

[illegible] ne, but 'hyper-
ction, which is

DE DUVE It was not of course suspected to be a hormone when it was first described.

YOUNG *Although we may not all agree, perhaps we should adopt* the name 'glucagon' for use during this meeting

BEST I should like more information on the origin of glucagon For example, in what part of the gastro-intestinal tract is it found? Would Professor de Duve please review this for us?

DE DUVE Large amounts of glucagon are found in the upper two-thirds of the gastric mucosa of the dog and small amounts in the duodenum. There is very little in the pyloric part of the stomach and none in the colon or gall-bladder There is some in rabbit stomach but none in pig or ruminant stomach and practically none in guinea-pig stomach. This distribution corresponds roughly to that of a special type of *argentophil cell described by Tehver However, there are* silver-staining cells in many organs which do not contain glucagon Silver staining is a very unspecific method It seems possible, embryologically, that some cells identical with the alpha-cells may be scattered along the digestive tract in some species The pancreas is the only organ known to contain glucagon in all species

BEST In what species is it found *only* in the pancreas?

DE DUVE In the pig

LUKENS And this animal does not exhibit a typical diabetes!

LAZAROW There appear to be at least two types of silver-staining cells One type will reduce silver ions directly A second type will *adsorb colloidal silver but it is necessary to add a reducing agent to* form the colloidal silver micelles which are then adsorbed on the cells

LUKENS Is the amount of insulin remaining in the pancreas in alloxan diabetes known? Dohan and I had three dogs with pituitary diabetes in which the pancreases were intact and which excreted the same amount of nitrogen as depancreatized dogs We called this 'total urinary diabetes' By this criterion the diabetes was of the same severity whether or not the alpha-cells were present But both types of animals were fasted This suggests that there is no influence of glucagon during fasting

DE DUVE We found no significant change in the glucagon content of *the pancreas of guinea-pigs after fasting them for five days*

YOUNG Marks and I fed large amounts of raw pancreas in the diet of depancreatized dogs and analysed the faeces to make sure that the absorption of food was the same as in comparable dogs with meta-hypophyseal diabetes In one dog with metahypophyseal diabetes the insulin requirements fell after pancreatectomy although the food intake was the same (MARKS, H P and YOUNG, F G, *J Endocrin*, 1, 470, 1939) The difference in insulin requirement was not due to a

D

difference in absorption of food from the gut, but might have been due to the liberation of glucagon from the pancreas in the dogs with metahypophyseal diabetes

LUKENS These effects have not always been observed Machella found that the insulin requirement of a totally depancreatized man rose from 40 to 80 units when a different pancreatic enzyme preparation was administered In depancreatized men there also seems to be a great difference according as to whether or not diarrhoea occurs The situation seems to be very complex.

YOUNG These results are not incompatible with those of Marks and Young

[illegible]

usually decreased the requirement for insulin In other dogs with metahypophyseal diabetes, however, pancreatectomy had little effect on the insulin requirement An adequate amount of raw pancreas was included in the diets of these dogs, both before and after pancreat- [illegible]

LAZAROW With regard to Mirsky's experiments [illegible] protein in the faeces represents unabsorbed protein A considerable *part of the protein content of the faeces is bacterial in origin and the* absence of the alkaline pancreatic juice may affect the bacterial flora of the gastro-intestinal tract I recently discussed this point with Mirsky and we agreed that it would be better to eliminate the absorption factor He plans to compare the severity of the diabetes in alloxan treated and in depancreatized dogs when glucose and amino-acids are injected intravenously

LUKENS Has Professor Candela studied the absorption of food in connection with the work reported in his paper?

CANDELA In animals made diabetic with alloxan, we waited till the diabetes was stabilized, then tied the pancreatic ducts, and finally, after some weeks, removed the pancreases The diet was raw pancreas plus 'Pankreon' *Immediately after pancreatectomy*, the insulin requirement changed but the weights of the animals remained constant There were no signs of malnutrition

DE JONGH The insulin sensitivity of alloxan treated rats is very low, not only if compared with depancreatized ones, but also if compared with normal animals We considered the possibility of there being an enhancement of glucagon production after treatment with alloxan, but from Professor de Duve's work we concluded that this is not

enlarged in alloxan diabetic animals Glucagon injected intravenously causes a significant decrease in the eosinophil count in the dog Injection of the solvent alone has no effect but injection of glucose in amounts sufficient to raise the blood sugar to the same hyperglycaemic level as obtains with glucagon causes a similar decrease in the eosinophil count We do not know therefore whether the effect of glucagon is specific

Long Did you study the effect in adrenal-demedullated dogs in case glucose itself stimulated the secretion of epinephrine? One must be very careful because the eosinophil count appears to be very sensitive to painful or other stimuli that may occur after subcutaneous or intraperitoneal injections

de Duve We did not do this

Long Injection of a few drops of 10 per cent sodium chloride solution results in a marked drop in the eosinophil count due to the pain caused by the injection

de Duve In our experiments the difference from the controls was merely one of a few micrograms of protein as glucagon

Young Did you do control experiments with even a few micrograms of a non-specific protein? Vogt has I believe observed a marked effect of non-specific protein on the eosinophil count

de Duve No We would have to check this

Long Is the adrenal ascorbic acid concentration affected?

de Duve This was not studied

Young Have you any comments on the purity of glucagon preparations Professor de Duve?

de Duve Perhaps Professor Cori has for the work has been done in his laboratory

Carl Cori We have done no further work on purification This is being carried out by Eli Lilly & Company Electrophoresis gave no indication how much insulin was present

de Duve Sutherland Cori Haynes and Olsen whose method has been used in our experiments have achieved a tenfold purification of glucagon. They describe a second electrophoretic component in glucagon-containing insulins which amounts to approximately ten per cent of the total protein If this component represents glucagon their samples and ours must be fairly pure

Young Our specimens of glucagon are all very impure all the preparations tested contained some insulin

RUSSELL Perhaps glucagon is present in the pancreatic extracts used for the assay of insulin This might give rise to some error

DE DUVE This is a very important point Glucagon is quite active when given subcutaneously and must antagonize or delay the action of insulin injected simultaneously

RUSSELL Perhaps the duck has more insulin than is thought then

BEST I agree Dr Vuylsteke and Professor de Duve might perhaps estimate both the insulin and the glucagon in duck pancreas as they have in guinea-pig pancreas (See footnote to Professor de Duve's paper—*Ed*)

HAGEDORN If one compares the mouse and the rabbit methods of [illegible] nd that the mo[illegible]se test is less sens[illegible]e to gl[illegible]cagon [illegible] for insulin with the mouse?

HAGEDORN Yes

YOUNG Therefore differences by the two methods may be due to the presence of glucagon

HAGEDORN With mixtures there may be other complications due, for example to different rates of absorption from the subcutaneous tissue

DE DUVE This point could be checked since glucagon is inactivated by trypsin much more quickly than insulin This method is used by Eli Lilly & Company to prepare insulin free from ascertainable glucagon activity

YOUNG There may be still some glucagon in insulin which appears to be pure by biological tests—even by the liver slice test

DE DUVE I agree

ALLOXAN DIABETES AND THE MECHANISM OF BETA-CELL DAMAGE BY CHEMICAL AGENTS

By

ARNOLD LAZAROW

Department of Anatomy, Western Reserve University School of Medicine, Cleveland, Ohio, U S A

INTRODUCTION

The finding by Dunn, Sheehan and McLetchie that alloxan produces selective necrosis of the beta-cells in the islets of Langerhans of the pancreas (23) has been an important landmark, for this compound has provided a simple method of producing experimental diabetes in animals (3, 22, 26). Alloxan has proved to be diabetogenic in most species studied (57), including man [illegible]

can be detected within a few minutes of the alloxan injection Within twenty-four hours marked damage is evident—the beta-cells become shrunken and rounded, the nucleus becomes pyknotic and the cytoplasm loses its specific granulations Within a week most of the beta-cells have disappeared leaving little evidence of fibrosis or scarring in the islet tissue In species where the beta-cells are localized in the central portion of the islet, only a complex capillary network remains in this region By the use of special stains it may be shown that these islets are then composed almost exclusively of alpha-cells

Following an initial triphasic blood sugar response, a permanent hyperglycaemia develops as a consequence of the insulin deficiency (57) The animals show severe loss of weight, polydipsia, polyuria and glycosuria

PRODUCTION OF DIABETES WITH CHEMICAL AGENTS

The major classes of diabetogenic compounds and their relative effectiveness in producing beta-cell necrosis are shown in Table I

TABLE I

The production of diabetes with chemical agents

(i v intravenous administration i p intraperitoneal administration.)

Class of diabetogenic agent	Production of Diabetes: Dose per day per kg body wt (mg)	Production of Diabetes: Dose per day per kg body wt (mM)	Production of Diabetes: Days Required	Production of Diabetes: Conditions of administration	Species studied	Beta-cell changes	Characteristics of diabetes
Alloxan	40 i v	0 25 i v	1	starvation increased sensitivity	man dog cat rat mouse fish frog monkey duck etc	early extensive necrosis	smaller doses→ transitory diabetes
	200 i p	1 25 i p	1			late almost complete disappearance	larger doses→ severe permanent diabetes
Uric acid	1000 i p	6 0 i p	1 2	only in glutathione deficient rabbit	rabbit (not effective in rats)	necrosis	transitory diabetes (permanent diabetes in one rabbit only)

Dehydro-ascorbic acid	1100 i v	6 3 i v	1	requires small preliminary priming dose	rat rabbit	single dose→ slight changes	single dose→ transitory diabetes
	700 i v	4 0 i v				large dose→ necrosis	repeated dose→ permanent diabetes
Acetoacetic acid	50 i p increasing to 300 i p	0 5 i p increasing to 3 0 i p	30–90		rabbit rat	moderate changes	progressive fasting hyperglycaemia and decreasing glucose tolerance, slight glycosuria in one rabbit only
8-Hydroxy-quinoline	50 i v to 60 i v	0 36 i v to 0 43 i v	1		rabbit	extensive necrosis	smaller doses→ transitory hyperglycaemia
							larger doses→ permanent diabetes
Diphenylthio-carbazone	100 i v	0 38 i v	1		rabbit	extensive necrosis	smaller doses→ transitory hyperglycaemia
							larger doses→ permanent diabetes

TABLE I

The production of diabetes with chemical agents

(i.v. intravenous administration; i.p. intraperitoneal administration.)

Class of diabetogenic agent	*Production of Diabetes* — *Dose per day per kg body wt* (mg.)	(mM.)	*Days Required*	*Conditions of administration*	*Species studied*	*Beta-cell changes*	*Characteristics of diabetes*
Alloxan	40 i.v.	0.25 i.v.	1	starvation increased sensitivity	man, dog, cat, rat, mouse, fish, frog, monkey, duck, etc.	early extensive necrosis	smaller doses→ transitory diabetes
	200 i.p.	1.25 i.p.	1			late almost complete disappearance	larger doses→ severe permanent diabetes
Uric acid	1000 i.p.	6.0 i.p.	1–2	only in glutathione deficient rabbit	rabbit (not effective in rats)	necrosis	transitory diabetes (permanent diabetes in one rabbit only)

Dehydro-ascorbic acid	1100 i.v.	6.3 i.v.	1	requires small *preliminary* priming dose	rat rabbit	single dose→ slight changes	single dose→ transitory diabetes
	700 i.v.	4.0 i.v.				large dose→ necrosis	repeated dose→ permanent diabetes
Acetoacetic acid	50 i.p. increasing *to* 300 i.p.	0.5 i.p. increasing to 3.0 i.p.	30–90		rabbit rat	moderate changes	progressive fasting hyperglycaemia and decreasing glucose tolerance, slight glycosuria in one rabbit only
8-Hydroxy-*quinoline*	50 i.v. *to* 60 i.v.	0.36 i.v. to 0.43 i.v.	1		rabbit	*extensive* necrosis	smaller doses→ transitory hyperglycaemia
							larger doses→ permanent diabetes
Diphenylthio-*carbazone*	100 i.v.	0.38 i.v.	1		rabbit	extensive necrosis	smaller doses→ transitory *hyperglycaemia*
							larger doses→ permanent diabetes

Alloxan and related compounds The diabetogenic effects of compounds related to alloxan have been reviewed (57) It has been shown that methylalloxan and ethylalloxan also produce diabetes (12) (see Fig 1) Alloxantin which readily decomposes

ALTERATIONS WHICH DO NOT DESTROY THE DIABETOGENIC PROPERTIES	ALTERATIONS THAT DESTROY THE DIABETOGENIC PROPERTIES
ALLOXAN	ALLOXAN
8 HYDROXY QUINOLINE	8 HYDROXY-QUINOLINE
DIPHENYLTHIOCARBAZONE	DIPHENYLTHIOCARBAZONE

FIG 1

Diabetogenic properties and chemical structure

to give alloxan is also diabetogenic, whereas dialuric acid a simple reduction product of alloxan does not produce diabetes

[illegible]

within the body are reported to produce cytological evidence of beta-cell damage (25, 81), but clear-cut permanent diabetes has not been produced with these compounds (12, 27). Since uracil has limited solubility, we have injected large doses of its soluble riboside, uridine, but we have found that this compound does not produce clinical diabetes (45).

Uric acid. A massive dose of uric acid has been reported to produce diabetes when injected into glutathione-depleted rabbits (29). The animals were placed on a diet deficient in cystine and methionine prior to injection. This procedure low-

[illegible]

uric acid may be converted to alloxan within the body has been reviewed (44). However, recent attempts to demonstrate such a conversion *in vitro* using homogenates of rat liver have been unsuccessful (31). Similar studies have not been made using rabbit tissue.

Dehydroascorbic acid. The first three carbon atoms of dehydroascorbic acid have a chemical configuration which is almost identical with the mesoxalic portion of the alloxan molecule (2). Ascorbic acid has the same relationship to dehydroascorbic acid as dialuric acid has to alloxan. It is not surprising therefore that dehydroascorbic acid produces diabetes (64), whereas ascorbic acid, even when injected in doses of 5 g. per kg. body weight, does not produce diabetes (66). The diabetogenic dose of dehydroascorbic acid is very large compared with that of alloxan (see Table 1) and it is necessary to give this compound on three

[1] Although it has been reported that the injection of dialuric acid does produce diabetes (12), it is likely that the diabetes results from the oxidation *in vivo* of dialuric acid to alloxan. We have found that the injection of cysteine plus dialuric acid or of cysteine plus alloxan abolishes the diabetogenic effectiveness of both of these compounds. Since cysteine reduces alloxan to dialuric acid and also keeps the dialuric acid in the reduced state, the cysteine protection studies clearly indicate that dialuric acid *per se* is not diabetogenic unless it is oxidized to alloxan (52).

successive days in order to produce a permanent diabetes Furthermore, the diabetogenic dose of dehydroascorbic acid is much greater than the initial median lethal dose of this compound However, following the administration of a small dose of dehydroascorbic acid, a second dose, much larger than the median lethal dose will be tolerated Dehydro*iso*ascorbic acid (64) and dehydroglucoascorbic acid (65) also produce diabetes in rats

Acetoacetic acid This compound is reported to produce a progressive impairment in the glucose tolerance and to increase the fasting blood sugar level when injected daily in progressively *increasing amounts* (61) *However, only one of twenty* rabbits so injected developed a very mild glycosuria Repeated injections of acetone bodies are also reported to produce cytological damage in the beta-cells (60)

Oxidizing agents Compounds such as glyoxal (19) and ninhydrin have been studied for their diabetogenic effects Although these compounds do not produce clinical diabetes (12, 38), they are reported to produce cytological evidence of beta-cell damage (19, 78)

Chelating agents In their original communication on the production of diabetes, Dunn Sheehan and McLetchie reported that a styrylquinoline derivative was effective in producing diabetes in rabbits (23) More recently Kadota has reported that 8-hydroxyquinoline and diphenylthiocarbazone produced permanent diabetes by selectively destroying the beta-cells (41) Although Kadota originally suggested that the diabetogenic action of these agents was due to their ability to combine with zinc (41), his later studies with other chelating agents make this supposition more doubtful (42)

CHEMICAL PROPERTIES OF THE VARIOUS DIABETOGENIC AGENTS

Oxidation of sulphydryl groups Alloxan, by virtue of its keto group in position 5 (see Fig 2), is a very reactive compound It oxidizes cysteine and glutathione (GSH) to cystine and to oxidized glutathione (GSSG) respectively, the alloxan becomes reduced to dialuric acid (2, 52) Dehydroascorbic acid also oxidizes sulphydryl groups (80)

Reaction with glutathione In addition to oxidizing reduced glutathione (GSH) to its disulphide derivative (GSSG), alloxan

reacts with glutathione to form a new compound which we believe to be an addition product (69) This reaction takes place most rapidly at neutral pH and it produces a compound which has a maximum in its absorption spectrum at 305 mμ

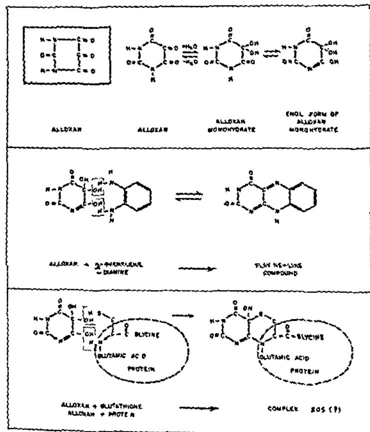


Fig 2
Reactions of alloxan

The ultraviolet absorption spectrum of this glutathione-alloxan reaction product can be readily differentiated from that of dialuric acid (maximum at 275 mμ) and from alloxan (maximum at 245 mμ) (69) Once formed, compound '305' is stable at

there are similarly two potentially reactive groups adjacent to each other

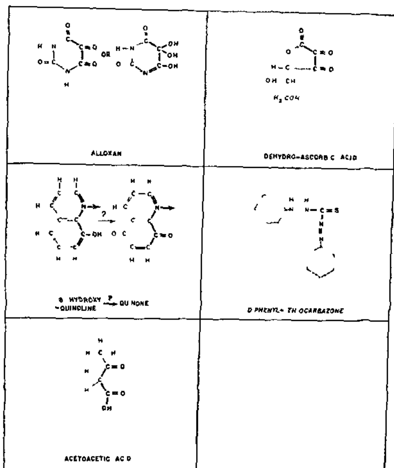


Fig 3
Possible active groups in diabetogenic compounds

Fig 3 shows the chemical groupings of the various diabetogenic agents That portion of the molecule which is most likely to be concerned with the diabetogenic properties is emphasized in bold lettering It would appear that all of these diabetogenic compounds have two active or potentially active groups which are closely adjacent to each other Even acetoacetic acid, which

is a weakly diabetogenic compound and which requires to be injected repeatedly to produce diabetes, has some structural similarities to the other compounds listed.

SELECTIVITY OF CHEMICAL AGENTS FOR THE BETA-CELLS

hemical
r these
to be
tewhat

greater than that required to produce diabetes will cause necrosis of the liver, kidney and other tissues (57) Still larger doses of alloxan will rapidly cause death It is likely that alloxan kills the cells because it directly or indirectly inactivates some critical enzyme or enzymes within the beta-cells It must be assumed that this critical enzyme which is inactivated by alloxan is not restricted to the beta-cells and is therefore not one of the specialized enzymes concerned with insulin synthesis, or else how could we account for the necrosis of the liver and kidney? If then, alloxan inactivates a critical enzyme which is present in many, or even in all cells, why does it selectively kill the beta-cells?

A localized accumulation within the beta-cells might occur with a less reactive diabetogenic agent, but it hardly seems likely in the case of alloxan The half-life of alloxan at 37°C and pH 7 4 is less than one minute (68) and the concentration of alloxan in the blood is reduced to below its diabetogenic level within the first five minutes of its injection (28) When the pancreatic artery of a dog was clamped for the immediate five-minute period following an alloxan injection, the beta-cells in the clamped portion of the pancreas showed no pathological changes, whereas that part of the pancreas which did not have its blood supply occluded showed typical beta-cell necrosis (28) Furthermore, dogs thus treated did not develop diabetes It therefore seems unlikely that selective accumulation of alloxan within the beta-cells could occur within the first minute or two of its injection

organ, this in turn determines the distance that the compound must diffuse before it enters the cell The amount of alloxan

reaching a highly vascular structure such as an islet of Langerhans is undoubtedly greater than that reaching less vascular structures such as resting muscle However, in many species the alpha-cells are interspersed among the beta-cells within the epithelial cords of the islet and yet the former cells are unaffected by diabetogenic doses of alloxan

It would therefore seem [illegible]

associated with (a) a low concentration of a critical enzyme within the beta-cell, (b) a lessened ability of the beta-cells to destroy or detoxify diabetogenic agents, or (c) a diminished concentration of a protective (antidiabetogenic) compound within the beta-cell

BIOCHEMISTRY OF THE BETA-CELL

In mammalian species the beta-cells are concentrated in a million or more islets of Langerhans scattered throughout the acinar tissue of the pancreas Since these cells constitute but a small fraction (one per cent) of the total weight of the pancreas, studies on the whole pancreas would give us little information about the metabolism of the beta-cells themselves In certain fish, however, the exocrine pancreas is separated from the islet tissue and the latter is concentrated into one or more discrete bodies known as the principal islets (18 73) But even in these species the islets contain several types of cells (40), and enzymic studies on the whole islets will give a weighted average for the metabolism of all of the component types of cells present It may, however, be possible to obtain more specific information about the individual types of cells by analysing islet tissue obtained from fish in which either the alpha-cells or the beta-cells (46) have been selectively destroyed (Selective necrosis of the alpha-cells has recently been produced by the injection of diethyldithiocarbamate (42))

Oxidative enzymes and intermediary metabolism Dr Cooper-[illegible] of the metabolism of

liver, kidney and many other tissues (48) Since the oxidation of succinic acid to fumaric acid (by succinic dehydrogenase) is an important step in the tricarboxylic acid cycle (34), it is probable that the other enzymes of the tricarboxylic cycle are also present in islet tissue, for if one of the enzymes of the cycle is present, it is likely that other components are also present Recently a histochemical method has been developed for determining the [illegible] The method [illegible] blue formazan dye is precipitated at the site of enzyme action Application of this histochemical method to the study of mammalian pancreas has shown that the succinic dehydrogenase content of islet tissue is lower than that of acinar tissue (7) We have also found that cytochrome oxidase is present in islet tissues (48) This likewise suggests that the other components of the cytochrome system are also present

The studies reported by R R. Bensley in 1911 on supravital staining of the pancreas with Janus green (10) may also give some indication of the enzyme systems present within the islet tissue Upon perfusion of a guinea-pig with a 1 10,000 solution of Janus green B, the entire pancreas was initially stained. When pieces of the pancreas were removed and placed under partially anaerobic conditions, the Janus green was reduced, first in the acinar cell cytoplasm and next in the acinar cell mitochondria By contrast, the islets of Langerhans were stained an intense blue for a long time after the dye had been completely decolourized in the rest of the pancreas

In order to learn more about the significance of this reaction we have studied the mechanism of Janus green staining (15, 47, 47a) We have shown that this blue dye is reduced enzymically to *leuco*-Janus green *Leuco*-Janus green is further reduced to the red dye safranin (by splitting of an azo bond) and finally to the colourless *leuco*-safranin Reduced flavoprotein (Straub) is the immediate enzyme which is capable of carrying out these reductions (15) Reduced diphosphopyridine nucleotide (DPNH) alone will not reduce Janus green even though it is present in substrate amounts, if, however, we add catalytic amounts of flavoprotein, the Janus green becomes reduced and the DPNH

becomes oxidized Under appropriate conditions of oxygen tension, and in the presence of flavoprotein, any dehydrogenase which is capable of reducing DPN could reduce Janus green The *leuco*-Janus green which is formed in the course of the reduction can be reoxidized to its blue derivative (Janus green) by molecular oxygen There is good evidence to suggest that the reoxidation of *leuco*-Janus green within the living cell is catalysed by a cyanide sensitive, oxygen-dependent enzyme system which we believe to be cytochrome oxidase (47, 47a) Thus the supravital staining with Janus green *is dependent upon* a balance between the dehydrogenase-DPN-flavoprotein system

therefore be taken as evidence suggesting that, of the various dehydrogenases, islet tissue is relatively deficient in cytochrome oxidase We have found, by direct enzymic study, that the ratio of succinic dehydrogenase to cytochrome oxidase in fish islet is low compared with that in most of the other tissues studied (48) Although succinic dehydrogenase does not reduce DPN directly —and therefore the relative concentration of this enzyme is not a measure of the ability of the cell to reduce Janus green—the low ratio of succinic dehydrogenase to cytochrome oxidase may be a reflection of the general metabolic specialization of islet tissue

Glucose and beta-cell function We are studying the metabolism of glucose by islet tissue (49) and we plan to look for the specific *enzymes that are involved in glucose utilization* The effect of glucose on the function of the beta-cell is of special interest for there is good evidence to show that the output of insulin by the beta-cells is controlled by the concentration of glucose bathing the cell When the intact (86) or the isolated (1) *pancreas is perfused* with a fluid containing hyperglycaemic levels of glucose, the beta-cells respond by increasing their output of insulin

blood sugar le

secretion of insulin could be due

insulin (that is, a degranulation of the beta-cells) or to the synthesis of new insulin, or both It might be of considerable practical

importance to determine the mechanism by which the glucose concentration controls the synthesis or release of insulin from the beta-cell.

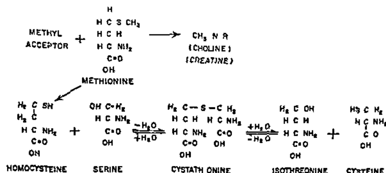
Protein synthesis The primary function of the beta-cell is the continued synthesis of insulin The approximate rate of insulin synthesis in man was calculated to be about 2 mg of insulin protein per day (assuming that a normal man requires about 60 units of insulin per day and that 1 mg of insulin is equal to 28 units) The rate of insulin synthesis by the beta-cells was calculated to be about 30 µg. of protein per milligram of tissue (dry weight) per day (In making this calculation it was assumed that one per cent of the weight of the pancreas (75 g) is islet tissue, that one half of the islet is composed of alpha-cells, sinusoids and connective tissue, and that beta-cells contain about eighty per cent water) Thus about four per cent of the protein within the beta-cell would be synthesized anew each day as insulin (assuming that three-quarters of the dry weight of the cell is protein)

It may be presumed that the essential amino-acids are taken up by the beta-cells, incorporated into peptide bonds and synthesized into the insulin molecule Considerable energy is required for the synthesis of peptide bonds, for it has been calculated that one mole of adenosine triphosphate (ATP) is needed for each mole of peptide bond synthesized (54) This energy must, of course, be derived from the oxidation of other foodstuffs The *minimum* amount of energy required for synthesizing the peptide bonds of insulin has been calculated by assuming that the average molecular weight of the amino-acids in insulin is 120 The *minimum* amount of oxygen utilized in the synthesis of the daily insulin requirement (assuming that one mole of ATP is equivalent to one-sixth of a mole of oxygen, *i e*, 3,740 ml. at N T P) was calculated to be 1 0 microlitre of oxygen per milligram of beta-cells (dry weight) per day On an hourly basis it is 0 04 µl /mg /hr Thus the *minimum* amount of energy required for the synthesis of the peptide bonds of insulin is probably only a small fraction of the total energy liberated by metabolism in the beta-cell

Although little is known about the enzymes that are involved in protein synthesis, the recent work of Hanes et al suggests that

transpeptidation of amino-acids may be an important reaction in protein synthesis (33) Further, their work indicates that glutathione plays a role in this transpeptidation reaction

The synthesis of *insulin by the beta-cells is undoubtedly* dependent upon a continuous supply of essential amino-acids,



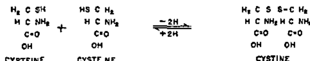


Fig 4

The biosynthesis of cysteine

for when rabbits are placed on a diet deficient in cystine and methionine, the insulin content of the pancreas is decreased (29) The thio-amino-acids may be of critical importance to insulin *synthesis for although methionine* is absent from insulin (84) this protein contains an unusually large amount (12 per cent) of cystine (82)

The nutritional requirements for the *thio-amino-acids may* ... Since methionine ...d in ... are synthesized from methionine The stages in the synthesis of cysteine are outlined in Fig 4 Methionine is converted to homo-

cysteine by demethylation The homocysteine reacts with serine to form the addition product cystathionine (11) which then gives rise to cysteine by hydrolysis Thus the sulphur of methionine is incorporated into cysteine whereas the rest of the cysteine molecule is synthesized anew Cysteine is a sulphydryl-amino-acid and when this amino-acid is oxidized two molecules are joined together by a disulphide bridge All of the sulphur in insulin is in the disulphide state (82) Indeed, if the disulphide

of sulphydryl groups and there may be reason to think that they have a special metabolic pathway to carry out this oxidation It is not known whether the sulphydryl-amino-acids are incorporated into the polypeptide chain and later oxidized to disulphide or whether the disulphide-amino-acids are incorporated as such Sanger has clearly shown that insulin is made up of

tide chain as the disulphide-amino-acid, then peptide synthesis would have to proceed simultaneously along all four of the component polypeptide units If, on the other hand, the sulphydryl-amino-acids were incorporated into the polypeptide chain then each component polypeptide chain could be synthesized independently, these could then be joined together after the synthesis of the component units were completed (see Fig 5)

The protein-bound sulphydryl groups in islet tissue Barrnett and Seligman (8) have recently described a histochemical reaction which can be used for the detection of protein-bound sulphydryl groups The proteins are fixed and denatured with trichloroacetic acid (This renders the proteins insoluble and liberates the so-called masked' sulphydryl groups) The specificity of this procedure is based on the reaction of the protein sulphydryl group with the disulphide bond of the histochemical reagent (2 2-dihydroxy-6 6-dinaphthyl disulphide) As a result, an insoluble protein disulphide complex (2-hydroxy-6-naphthyl protein disulphide) is formed In the second stage of the histo-

chemical procedure, a coloured azo dye is formed by coupling the hydroxynaphthyl derivative with tetrazotized di-*ortho*-anisidine The resulting protein disulphide azo dye is insoluble in water, ether and alcohol whereas the unreacted reagent and the other reaction products are soluble and may be removed by washing The sites of protein-bound sulphydryl groups stain red or blue The histochemical reaction is abolished by the prior treatment of the tissue sections with iodine, which is known to oxidize sulphydryl groups Application of this technique to

FIG 5
The synthesis of insulin and glutathione by the beta-cell

mammalian pancreas has shown that islet tissue contains relatively few protein-bound sulphydryl groups (8) The islets appear as islands of practically unstained tissue surrounded by the more deeply stained acinar tissue Insulin is dissolved from the cell under the conditions of this histochemical reaction (8) but since insulin contains no sulphydryl groups (82) it would not account for any of the protein bound sulphydryl groups detected within the intact beta-cell

Glutathione content of the beta-cell Although glutathione is present in the blood cells, it is absent from the serum (55) and therefore this substance is not readily diffusible It is probable that glutathione is synthesized -cell Since cysteine (or cystine) for the and glutathione (see may be for this amino-acid

within the beta-cell By assuming that the glutathione concentration in the beta-cell equals that in the whole pancreas, we have calculated that if all of the cysteine contained in the glutathione of the beta-cell were to be incorporated into insulin, then only

Role of glutathione in protecting sulphydryl enzymes It has been shown that the biological activity of a large number of enzymes depends upon the presence of active sulphydryl groups (9) These include enzymes which are important in the metabolism of carbohydrates, fats and proteins When essential sulphydryl groups of an enzyme are blocked by adding an appropriate sulphydryl reagent the enzyme becomes inactive If the sulphydryl group in the enzyme can be liberated again the enzyme becomes reactivated. Sulphydryl groups can be blocked by a number of different reagents (36, 37) Compounds such as mercury and arsenic form mercaptides with sulphydryl groups this reaction is reversible Mild oxidizing agents convert sulphydryl groups

can be

ry the

ge and

various sulphur acids (sulphenic, sulphinic, sulphonic) are formed

which contains a sulphydryl group Glutathione will protect

om-

can

the

disulphide form and can remove arsenic and mercury from combination (39) However, it cannot reduce sulphydryl groups oxidized past the disulphide stage, nor can it reverse the effect of some alkylating agents

Glutathione as a coenzyme Glutathione is a coenzyme for

More recently, Racker and Krimsky have found that glutathione reacts as a coenzyme for triosephosphate dehydrogenase (72) The glutathione is firmly bound to the protein and it is liberated from the enzyme by heat denaturation and acid hydrolysis It is believed that DPN, which is a second coenzyme for this reaction, combines with the sulphydryl group of the gluta-

then cleaved in the presence of phosphate, forming 1 3-di-

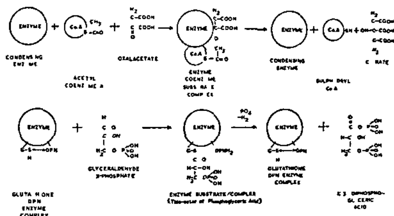


Fig 6

Reactions dependent on glutathione and coenzyme A.

phosphoglyceric acid Thus glutathione plays an important role in the oxidation of glyceraldehyde-3-phosphate

Glutathione also plays a role in some transacetylation reactions Glyceraldehyde-3-phosphate dehydrogenase catalyses the transfer of acetyl groups from acetyl phosphate to glutathione and a product with the properties of *S*-acetylglutathione has been isolated (35, 72) In the presence of glyceraldehyde-3-phosphate dehydrogenase, glutathione coenzyme A, oxaloacetate and the condensing enzyme, transacetylation takes place, acetate con- … acetate to form citric acid (35) It is of interest

ester When acetylated coenzyme A reacts with oxaloacetate,

one mole of sulphydryl is liberated for each mole of citrate synthesized (77) (see Fig 6)

Glutathione also plays a role in transpeptidation reactions (33)

POSSIBLE MECHANISMS BY WHICH ALLOXAN AND OTHER CHEMICAL AGENTS KILL BETA-CELLS

Inactivation of glutathione The role of glutathione as a coenzyme [illegible]

the glutathione content of the blood (53) and tissues (17) We have found that glutathione, when injected in doses equivalent to the amounts found in the body, completely protects rats against a diabetogenic dose of alloxan (43) or dehydroascorbic acid (66)

[illegible]

phosphate by triosephosphate dehydrogenase is a necessary step in the oxidation of glucose (70) (see Fig 7) Similarly, transacetylation reactions dependent on coenzyme A are important in the oxidation of carbohydrates, fats and certain amino-acids The 'active acetate' intermediates that are formed from these foodstuffs are converted by transacetylation to oxaloacetate and [illegible]

[illegible]

tion by chemical agents such as alloxan and dehydroascorbic acid For if the concentration of glutathione within a cell is limited, then the destruction of a major fraction of the glutathione would produce a severe metabolic defect On the other hand if the concentration of glutathione in a cell is in great excess of that required for a given enzymic reaction, then the destruc-

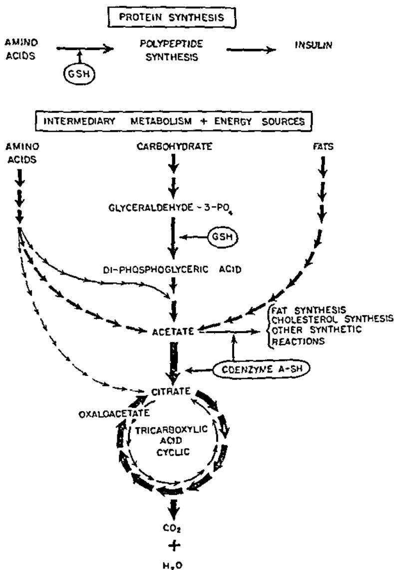


FIG. 7
Sulphydryl-dependent reactions

tion of an equivalent fraction of the glutathione might produce only minor alterations in the metabolic activity of that cell If the selective destruction of beta-cells is due to their low glutathione content, then larger doses of the diabetogenic agent would kill other cells in addition, and this has been found to be true with alloxan as the diabetogenic agent

Inactivation of enzymes We have also noted that non-specific

great excess of its needs, the inactivation of ninety per cent of the enzyme might not significantly interfere with the ability of that cell to utilize the amounts of the substrate which appear under physiological conditions On the other hand, if the concen-

has been observed following the injection of enzyme inhibitors (71)

Since many enzymes contain active sulphydryl groups (9) and since alloxan combines with the sulphydryl groups of protein (69) I have suggested that this diabetogenic agent may kill the beta-cells by directly inactivating enzymes (43) It has already been noted that the total concentration of the protein-bound sulphydryl groups within the islet tissue is low (8) and that a chemical agent which combines with protein sulphydryl groups might selectively destroy the beta-cells It is doubtful whether all of the protein-bound sulphydryl groups present in islet tissue are enzyme sulphydryl groups, nevertheless, when the concentration of non-essential (or non-specific) sulphydryl groups is low, more alloxan is then available to react with essential sulphydryl groups Since glutathione also protects sulphydryl enzymes (9), a low glutathione concentration would likewise contribute to the selective destruction of beta-cells

The production of beta-cell necrosis (and diabetes) by chemical agents that are capable of combining with trace metals (41) is of interest for it suggests that these agents might kill the cells by inactivating a critical metal-dependent enzyme The biological activity of many enzymes and coenzymes is dependent upon

the presence of various trace metals (79) For example, iron is a component of cytochrome oxidase and cytochrome *c*, whilst cobalt is a component of *vitamin* B_{12} Magnesium is necessary for carboxylase activity, copper for tyrosinase activity and zinc for carbonic anhydrase activity Since metal-dependent enzymes are present in most cells, it would likewise be necessary to attribute the selectivity of these agents to a low concentration of one or more metal-dependent enzymes within the beta-cell

Kadota, however, has already reported that many effective chelating agents do not produce diabetes (41, 42) There is reason, therefore, to wonder whether the diabetogenic effects of 8-hydroxyquinoline and diphenylthiocarbazone are due to their metal-binding properties

In experiments designed to study the effect of trace metals on the diabetogenic properties of alloxan, we injected the metal *intravenously*, *immediately before*, and *fifteen minutes after* a diabetogenic dose of alloxan (50) We found that cobalt (0 17 mM per kg), zinc (0 10 mM per kg) and ferrous iron (0 108 mM per kg) protected against diabetes when they were administered before the alloxan On a molar basis, cobalt was five times as effective as zinc in protecting against diabetes and the protective dose of cobalt was one-fifteenth of the dose of alloxan injected None of the metals, however, was capable of reversing the diabetogenic effects when given fifteen minutes after the alloxan If the diabetogenic effects of alloxan were due to combination with trace metals, then the diabetes should have been reversed by supplying the metal after the alloxan, unless, of course, a metal-alloxan complex remained firmly attached to the enzyme The fact that three different metals are capable of protecting against diabetes when they are given before the alloxan would suggest that the effect is non-specific These metals may combine with alloxan or catalyse its destruction, thereby lowering the effective concentration of alloxan in the beta-cell, or they may reversibly combine with the sulphydryl groups that react with alloxan and thereby afford an indirect protection

Selective beta-cell necrosis due to a decreased capacity to destroy or detoxify diabetogenic agents Diabetogenic agents such as alloxan, dehydroascorbic acid and diphenylthiocarbazone lose their diabetogenic potency when they are reduced to dialuric acid,

ascorbic acid and diphenylthiocarbazide respectively (see Fig 1) Therefore, the concentration of a diabetogenic agent within a cell is dependent not only upon the amount injected, but also upon the ability of the cell to reduce this compound to its non-diabetogenic derivative Alloxan can be reduced to its non-diabetogenic derivative by cysteine (2 69), by glutathione (2, 69) and by reduced coenzyme 1 (DPNH) (16) On the other hand, dialuric acid can be reoxidized to its diabetogenic derivative by oxidized cytochrome *c* (16) Dehydroascorbic and ascorbic acids have a similar interrelationship (80) Thus a low concentration of glutathione within the beta-cell, or a low concentration of the enzymes which are capable of reducing alloxan or dehydroascorbic acid to their non-diabetogenic derivatives would also contribute to a mechanism for selectively destroying beta-cells

The studies so far reported on the enzymes in islet tissue (48) may have a bearing on this point The ability of the beta-cell to reoxidize dialuric acid to its diabetogenic derivative would be determined not only by the absolute concentration of cytochrome *c* but also by the fraction of the cytochrome *c* that is in the oxidized state Although we have not as yet been able to determine the absolute concentration of cytochrome *c* within islet tissues, we have studied some of the enzymes which oxidize and reduce cytochrome c We have found that the concentration of succinic dehydrogenase in islet tissue is low and that the ratio of succinic dehydrogenase to cytochrome oxidase is also low

The proportion of the cytochrome *c* that is in the oxidized state would be determined by both the absoute and the relative concentrations of the enzymes that reduce and oxidize cytochrome *c* Structural factors which determine the spatial relationship of these enzymes to one another would also be important Cytochrome oxidase is the only known enzyme which is capable of oxidizing cytochrome *c* On the other hand, succinic dehydrogenase is one of a number of enzymes capable of reducing cytochrome *c* If the distribution in islet tissue of other enzymes which reduce cytochrome *c* parallels that of succinic dehydrogenase then a larger proportion of the cytochrome *c* which is present in islet tissue would be in the oxidized state This would

(and other sulphydryl groups) This could sufficiently impair the metabolic function of the cell to kill it

It has been suggested that the selectivity of diabetogenic agents for the beta-cell may arise from the cell's specialization for insulin synthesis The following factors have been considered as possible causes of the selective destruction of beta-cells by diabetogenic agents

(a) a low concentration of a critical enzyme or coenzyme (possibly glutathione) in the beta-cell,

(b) a low concentration of an antidiabetogenic or protective substance (glutathione) in the beta-cell,

(c) an inability or a diminished ability of the beta-cell to destroy or to detoxify diabetogenic agents

The possibility that diabetogenic agents may produce a functional impairment of insulin synthesis as a consequence of glutathione inactivation has been discussed

REFERENCES

1 ANDERSON, E and LONG, J A, *Endocrinology*, 40, 92 (1947)
2 ARCHIBALD, R M *J biol Chem*, 158, 347 (1945)
3 BAILEY, C C and BAILEY, O T, *J Amer med Ass*, 122, 1165 (1943)
4 BANERJEE, S, *Nature, Lond*, 153, 344 (1944)
5 BANERJEE, S, DEB, C and BELAVADY, B, *J biol Chem*, 195, 271 (1952)
6 BANERJEE, S and GHOSH N C, *J biol Chem*, 168, 207 (1947)
7 BARRNETT, R J, personal communication
8 BARRNETT, R J and SELIGMAN A M, *Science*, 116, 323 (1952)
9 BARRON E S G, *Advanc Enzymol*, 11, 201 (1951)
10 BENSLEY, R R, *Amer J Anat*, 12, 297 (1911)
11 BINKLEY, F and DU VIGNEAUD, V, *J biol Chem*, 144, 507 (1942)
12 BRUCKMANN, G and WERTHEIMER E, *J biol Chem*, 168, 241 (1947)
13 COLLINS-WILLIAMS, J and BAILEY, C C, *Proc Soc exp Biol, N Y*, 71, 583 (1949)
14 CONN, J W and HINERMAN, D H, *Amer J Path*, 24, 429 (1948)
15 COOPERSTEIN, S J and LAZAROW, A, *Biol Bull*, *Wood s Hole*, 99, 321 (1950)

16 COOPERSTEIN, S J, LAZAROW, A and PATTERSON, J W, unpublished experiments
17 DE CARO, L and ROVIDA, E, *Boll Soc ital Biol sper*, 12, 611 (1937)
18 DIAMARE, V, *Int Mschr Anat Physiol*, 15, 155 (1899)
19 DOERR, W, BOPP, F, KUHN, R and QUADBECK, G, *Naturwissenschaften*, 35, 125 (1948)
20 DRAKE, B B, SMYTHE, C V and KING, C G, *J biol Chem*, 143, 89 (1942)
21 DUFF, G L, *Amer J med Sci*, 210, 381 (1945)
22 DUNN, J S and MCLETCHIE, N G B, *Lancet*, ii, 384 (1943)
23 DUNN, J S, SHEEHAN, H L and MCLETCHIE, N G B, *Lancet*, i 484 (1943)
24 FROMAGEOT, C, *Advanc Enzymol*, 7, 369 (1947)
25 GITTER, S and CARDEZA, A F, *Rev Soc argent Biol*, 22, 353 (1946)
26 GOLDNER, M G and GOMORI, G, *Endocrinology*, 33, 297 (1943)
27 GOLDNER, M G and GOMORI, G, *Endocrinology*, 35, 247 (1944)
28 GOMORI, G and GOLDNER, M G *Proc Soc exp Biol, N Y*, 58, 232 (1945)
29 GRIFFITHS, M, *J biol Chem*, 184, 289 (1950)
30 GRIFFITHS, M, personal communication
31 GUNNERT, R R and PHILIPS P H, *J biol Chem.*, 191, 633 (1951)
32 HALL, B V and MAY R E, *Anat Rec*, 112, 36 (1952)
33 HANES, C. S, HIRD, F J R and ISHERWOOD, F A, *Nature, Lond*, 166 288 (1950)
34 HARROW, B, *Textbook of Biochemistry*, 4th ed, Philadelphia Saunders (1946)
35 HARTING J and VELICK, S, *Fed Proc*, 11, 226 (1952)
36 HELLERMANN, L, *Physiol Rev*, 17, 454 (1937)
37 HELLERMANN, L, CHINARD, F P and DEITZ, V P, *J biol Chem*, 147, 443 (1943)
38 HIDY, P H, *J biol Chem*, 163, 307 (1946)
39 HOPKINS, F G, *Biochem J*, 19, 787 (1925)
40 JACKSON, S, *J. metab Res*, 2, 141 (1922)
41 KADOTA, I, *J Lab clin Med*, 35, 568 (1950)
42 KADOTA, I, *J Lab clin Med*, 38, 671 (1951)
43 LAZAROW, A, *Proc Soc exp Biol, N Y*, 61, 441 (1946)
44 LAZAROW, A, *Physiol Rev*, 29, 48 (1949)
45 LAZAROW, A, unpublished experiments
46 LAZAROW, A and BERMAN, J, *Biol Bull*, *Wood's Hole*, 93, 219 (1947)

47 LAZAROW, A and COOPERSTEIN, S J, *Biol Bull*, *Wood's Hole*, 99, 322 (1950)

47a LAZAROW, A and COOPERSTEIN, S J, *Exp Cell Res*, in the press

48. LAZAROW, A and COOPERSTEIN, S J, *Biol Bull*, *Wood's Hole*, 100, 191 (1951)

[illegible]

52 LAZAROW, A, PATTERSON, J W and LEVEY, S, *Science*, 108, 308 (1948)

53 LEECH, R S and BAILEY, C C, *J biol Chem*, 157, 525 (1945)

54 LIPMANN, F, *Fed Proc*, 8, 597 (1949)

55 LITARCZEK, G and DIMISCHIOUTI, G T, *C R Soc Biol*, *Paris*, 114, 285 (1933)

56 LOHMANN, K., *Biochem Z*, 254, 332 (1932)

57 LUKENS, F D W, *Physiol Rev* 28, 304 (1948)

58 MOLANDER, D W and KIRSCHBAUM, A, *Endocrinology*, 44, 391 (1949)

59 MORTON, A, *The Chemistry of Heterocyclic Compounds*, New York McGraw-Hill (1946)

60 NATH, M C, BRAHMACHARI, H D and GOPALKRISHNA, P A, *Science*, 112, 92 (1950)

61 NATH, M C and CHAKRABARTI, C H, *Proc Soc exp Biol*, *N Y*, 75, 326 (1950)

62 NATH, M C and HATWALNE, V G, *Nature*, *Lond*, 166, 692 (1950)

63 OCHOA, S, *Physiol Rev* 31, 56 (1951)

64 PATTERSON, J W, *J biol Chem*, 183, 81 (1950)

65 PATTERSON, J W, *Science*, 111, 724 (1950)

66 PATTERSON, J W and LAZAROW, A, *J biol Chem*, 186, 141 (1950)

67 PATTERSON, J W and LAZAROW, A, unpublished experiments

68 PATTERSON, J W, LAZAROW, A and LEVEY, S, *J biol Chem*, 177, 187 (1949)

69 PATTERSON, J W, LAZAROW, A and LEVEY, S, *J biol Chem*, 177, 197 (1949)

70 POTTER, V R and HEIDELBERGER, C, *Physiol Rev*, 30, 487 (1950)

71 POTTER, V R and SIMONSON, H, *Proc Soc exp Biol*, *N Y*, 76, 41 (1951)

72 RACKER, E and KRIMSKY, L, *Nature*, *Lond*, 169, 1043 (1952)

73 RENNIE, J, *Quart J micr Sci*, 48, 379 (1905)

74 SANGER, F, *Cold Spr Harb Symp quant Biol*, 14, 153 (1949)
75 SELIGMAN, A M and RUTENBURG, A M, *Science*, 113, 317 (1951)
76 SIGAL, A and KING, C. G, *J biol Chem*, 116, 489 (1936)
77 STERN, J R., OCHOA, S and LYNEN, F, *Fed Proc*, 11, 293 (1952)
78 STOLL, W, *Z Naturf*, 1, 592 (1946)
79 SUMMER, J B and SOMERS, G F, *Chemistry and Methods of Enzymes*, 2nd ed, New York Academic Press, Inc (1947)
80 SZENT-GYORGYI, A, *Biochem J*, 22, 1387 (1928)
81 VARGAS, L and MUNDT, E, *Bol Soc Biol*, *Santiago, Chile*, 5, 59 (1948)
82. DU VIGNEAUD, V, *J biol Chem*, 75, 393 (1927)
83 DU VIGNEAUD, V, *Cold Spr Harb Symp quant Biol*, 6, 275 (1938)
84 DU VIGNEAUD, V, MILLER, G L and RODDEN, C, *J biol Chem*, 131, 631 (1939)
85 WOOD, H G, *Physiol Rev*, 26, 198 (1946)
86 ZUNZ, E and LABARRE, J, *C R Soc Biol*, *Paris*, 96, 710 (1927)

DISCUSSION

YOUNG Have you been able to confirm the work of Nath and Hatwalne concerning the diabetogenic action of acetoacetic acid, Dr Lazarow?

LAZAROW Yes We have repeated some of their experiments in rabbits and although most of our animals did not show a progressive impairment of the tolerance to glucose, an occasional rabbit did develop an abnormal tolerance There is some reason for believing that the method of preparation of the sodium salt of acetoacetic acid (from the ethyl ester) may be an important factor

GRIFFITHS Is the complete cytochrome oxidase system present in fish islets?

LAZAROW In our studies using the isolated toadfish islet tissue, we have demonstrated that succinic dehydrogenase and cytochrome oxidase are present The method which we used for estimating succinic dehydrogenase depends on measuring the rate of reduction of oxidized cytochrome *c*, and that for cytochrome oxidase is based on measuring the rate of oxidation of reduced cytochrome *c*. We have also found that succinic acid is oxidized by islet tissues

GRIFFITHS Was cytochrome *c* always added?

LAZAROW Yes, but I am fairly certain that we would observe some oxidation of succinic acid in the absence of added cytochrome *c*. We

did find, however, that the activity of the succinic oxidase system is very low in islet tissue compared with most other tissues in the fish.

LOUBATIERES Have the fluorescence spectra of alloxan and its derivatives in both the presence and absence of *ortho*-phenylenediamine been studied, as well as the ultraviolet absorption spectra?

LAZAROW We have not studied the fluorescence spectra of alloxan or its derivatives, but I believe Archibald has studied the spectra of the reaction product of alloxan and *ortho*-phenylenediamine

LOUBATIÈRES Is there any similarity between the two types of spectra?

LAZAROW I do not know Alloxan itself may not show any fluorescence

DE DUVE Is there any *direct* evidence that alloxan combines with [illegible]

[illegible]

evidence that alloxan reacts with reduced glutathione and with the sulphydryl groups of protein under physiological conditions, that is [illegible]

[illegible]

diabetogenic dose of alloxan

WILHELMI Have you studied the stability of the complex of alloxan with glutathione?

LAZAROW Although the maximum rate of complex formation occurs at pH 7 4, the complex, once formed, is much more stable at alkaline pH On the other hand the complex is very unstable at acid pH, decomposing almost instantly This reaction has been adapted [illegible] tion of glutathione in blood however,

[illegible] similar reaction?

LAZAROW Dehydroascorbic acid reacts with glutathione in a similar fashion We have not, as yet, studied the reactions of the [illegible] This may not be easy, for some of [illegible] have ultraviolet spectra

[illegible] l diabetogenic substances share the same mechanism of action

LAZAROW I quite agree There may be more than one way of killing the beta-cells

WILHELMI Does a previous injection of glutathione protect against all diabetogenic substances?

LAZAROW Glutathione will protect against alloxan and dehydroascorbic acid diabetes *Maske and de Moore have found that cysteine* and BAL protect against diphenylthiocarbazone diabetes We planned to study the effect of sulphydryl compounds in 8-hydroxyquinoline diabetes but we had difficulty in consistently producing diabetes with this compound Mary Root has had a similar experience, but she has been able to increase the incidence of diabetes by injecting methylene blue prior to the 8-hydroxyquinoline Protection studies under these conditions would be more difficult to interpret

DE DUVE. *Could* you give a little more explanation of *your theory* of enzyme inhibition?

LAZAROW The amount of a given enzyme present within the cell is probably, in many instances, in great excess of that required for the utilization of the amounts of substrates that are available under physiological conditions *For example, Nachmansohn* has shown that cholinesterase is essential for nerve conductivity, nevertheless, the conductivity of the nerve cell is not affected until over ninety per cent of the cholinesterase has been inhibited Potter has studied the effect of adding varying concentrations of an inhibitor to tissues and shown that a given concentration of an inhibitor may completely inactivate an enzyme in one tissue but not affect the corresponding enzyme in other tissues To me, at least, these experiments clearly indicate that though an *enzyme may be present in many cells*, a *non-specific* inhibitor may selectively kill certain cells

CHANGES IN THE BLOOD SUGAR LEVEL DURING THE FIRST HOURS AFTER ALLOXAN INJECTION

By

J H GAARENSTROOM and P SIDERIUS[1]

Phamacologisch Laboratorium der Rijks-Universiteit, Bloemsingel 1, Groningen, The Netherlands

INTRODUCTION

The changes in the blood sugar level occurring during the first hours after administration of a diabetogenic dose of alloxan have been studied by quite a number of investigators in several species of animals In accordance with the original observations (2, 13, 26 and others) it is generally agreed that the injection results in a phase of hyperglycaemia in most animals after one to four hours have elapsed This is consistently followed by a [illegible] several hours [illegible] of [illegible] In [illegible] rty minutes after the administration and preceding the initial hyperglycaemia, has been observed in rats (34) and in dogs (21, 35)

Many attempts have been made to elucidate the nature of these initial effects of alloxan on the blood sugar level The hyperglycaemic phase is absent in hepatectomized and eviscerated animals (21), which probably means that the liver is essential to the effect It has also been reported that the hyperglycaemia is abolished by adrenalectomy (12, 17, 28) or destruction of the adrenal medulla (17) This suggests that alloxan causes secretion of adrenaline and a subsequent breakdown of liver glycogen. Corkill *et al* observed that blocking the sympathetic nervous system by ergotamine prevented the hyperglycaemia (10) These effects have not, however, been unanimously confirmed Houssay *et al* found that there was still a rise in the blood sugar level in dogs and toads after adrenalectomy (21) Iversen, Kirschbaum *et al* and Shipley and Beyer have all observed a hyperglycaemic phase in demedullated or sympathectomized

[1] Present address Royal College of Medicine Baghdad, Iraq

animals (25, 28, 34). Iversen concludes that the adrenal cortex rather than the medulla is responsible for the effect on the liver

The hypoglycaemic phase occurring five to ten hours after the administration of alloxan has been attributed to the damaged islet cells releasing all their stored insulin (22) Direct evidence for such a hypothesis is lacking, the production of an equivalent hypoglycaemia by administering an amount of insulin equal to that contained in the animal's pancreas is only indirect evidence Moreover, opinion is divided about the effect of alloxan in depancreatized animals Goldner and Gomori did not find hypoglycaemia in depancreatized dogs (19) Houssay *et al* and Wrenshall *et al*, however, observed a fall in the blood sugar level in a number of cases if the pancreas had been removed only thirty to sixty minutes before the alloxan administration, but no such fall if twenty-four hours or more had elapsed between the operation and the injection (21, 35) Banerjee failed to observe hypoglycaemia after partial pancreatectomy (4), but Foglia *et al* reported the usual decrease in the blood sugar concentration (cited in 32) In alloxan diabetic animals the hypoglycaemia is absent (5, 18, 27) Clamping the pancreatic ducts for a few minutes after the intravenous injection of rabbits or dogs with alloxan usually prevents the injury to the islets, but nevertheless a hypoglycaemia still occurs in a majority of the animals after three to nine hours have elapsed (1, 9)

What makes the nature of this hypoglycaemic phase especially obscure are the observations in animals depancreatized shortly before the administration of alloxan The situation becomes even more complex if it is realized that the fall of the blood sugar level always occurs in normal animals, but only occasionally in depancreatized ones. As a possible explanation of the 'extrapancreatic' mechanism of the effect, Wrenshall *et al* and Bhattacharya suggest that the hypoglycaemia may be the result of a reduced supply of glucose from the liver to the blood (7, 35). This would mean that the increased breakdown of liver glycogen which takes place during the hyperglycaemic phase is rather suddenly reversed. This seems somewhat unlikely At the moment no ready explanation of the hypoglycaemia occurring five to ten hours after injection of alloxan can be given

The initial hypoglycaemic effect has attracted little attention,

many authors having failed to observe the effect possibly because its duration is so short It may be, however, that an initial hypoglycaemic phase of longer duration and greater extent is partly or entirely masked by the hyperglycaemia which sets in at about the same time Such a possibility is important to us because we recently suggested that the normal secretion of insulin may be mediated by alloxan in some way (29) One objection to such a hypothesis is that administration of diabetogenic doses of alloxan fails to cause a rapid fall in the blood sugar level The main purpose of the experiments described below was to counter this objection

We thought it best to attack the problem by investigating the changes in the blood sugar level during the first two and a half hours after administration of alloxan, using both normal animals and animals in which the possibility of a hyperglycaemic response or of a release of insulin had been eliminated as completely as possible

MATERIAL AND METHODS

Male and female rats weighing 130–200 g were used in all the experiments Food was withheld for ten to sixteen hours before each experiment was started Blood samples for the determination of the sugar level were taken from the tail vein immec [illegible] jection [illegible] the metho [illegible] and Okamato (16)

Alloxan (4 mg per 100 g body weight) was injected intravenously into all the rats of the following groups, the number in each group being given in brackets

Normal diabetic (D) animals (normal animals rendered diabetic by the alloxan injection) [10],

Normal non-diabetic (N-D) animals (normal animals not rendered diabetic by the injection) [14],

Adrenalectomized animals, operated on four days previously [15],

Adrenalectomized animals, operated on one hour previously [11],

Sham-operated animals [9],
Demedullated rats (adrenal medulla removed four weeks previously) [8],
Diabetic rats (pretreated with alloxan (4 mg per 100 g, I V) three to six days previously) [9],
Non-diabetic rats (responding to alloxan pretreatment with less than 1 g per day sugar excretion) [7].

There were also 10 rats adrenalectomized four days previously which were not treated with alloxan

The aim of the adrenalectomy was to abolish the hyperglycaemic response to alloxan There are two ways by which this might be achieved (i) by the great reduction in the glycogen content of the liver usually seen after this operation, which will largely prevent the possibility of glucose being liberated by this organ, and (ii) by removal of the adrenaline which is perhaps the stimulus for the breakdown of glycogen To distinguish between these two possibilities, one group was adrenalectomized four days and another group only one hour before the injection Low liver glycogen levels are likely to be found in the former type of preparation and unlikely in the latter Moreover, the effect of alloxan was also studied in demedullated rats, which would allow a more exact assessment to be made of the significance of adrenaline secretion by the medulla in the alloxan effect The diabetic and non-diabetic animals were used to elucidate the possible role of insulin in the alloxan effect

RESULTS

The average blood sugar values obtained are shown in Fig 1

The blood sugar level in the normal animals underwent changes which differed according to the ultimate effect of the alloxan injection If no diabetes resulted (normal N-D animals) it rose immediately and then gradually declined. On the other hand the blood sugar level of the animals which became diabetic (normal D animals) fell slightly at first, subsequently rose, though not up to the highest level found in the first group, and then decreased slowly until after 150 minutes there was little difference from the value reached in the animals that did not become diabetic The differences between the values obtained after thirty minutes are significant ($p = 0.003$)

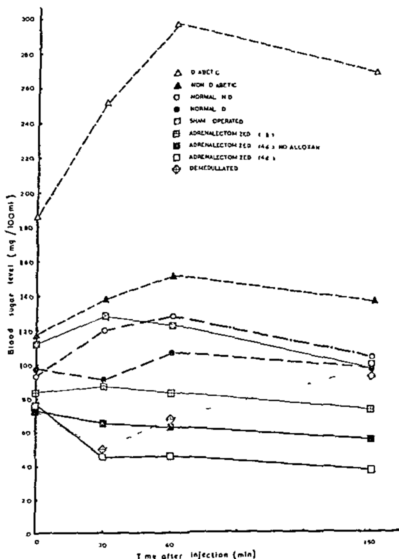


FIG 1

Blood sugar levels following intravenous administration of alloxan to rats (4 mg/100 g body weight)

In the animals adrenalectomized four days previously, alloxan caused a significant decline during the first thirty minutes (averages from 73 to 45 mg. per 100 ml, $p < 0.00006$). The decrease continued during the remaining two hours, though only slowly—perhaps because at such a low level a sharper decline is impossible The differences between the values after 30, 60 and 150 minutes in the adrenalectomized animals treated and not treated with alloxan are also significant. A number of adrenalectomized

alloxan injection, the initial level was higher than in the animals operated on four days previously and did not materially change during the experiment. In demedullated rats the blood sugar level fell initially and then rose until, after 150 minutes, it was about equal to that of normal animals

In diabetic animals the administration of alloxan caused an [illegible] The [illegible] ediate

COMMENT

As already mentioned, our aim was to find some confirmation of a hypoglycaemic effect of alloxan during the first hours after its administration We believe that these experiments do provide evidence for this It may perhaps be assumed for a moment that, under normal circumstances, the hypoglycaemia is partly or wholly obscured by the hyperglycaemia that is produced at about the same time. These two effects might almost neutralize each other during the first half-hour after the injection and minor factors will decide in any particular case whether hypo- or hyperglycaemia results.

If the hyperglycaemic response can be prevented, then the hypoglycaemia should be more easily demonstrable and this proved to be so in the rats adrenalectomized four days beforehand. In these animals the hyperglycaemia could not be produced because of the loss of liver glycogen that follows adrenalectomy, this explains the maintenance of the hypoglycaemia

which proved fatal in a number of cases In the demedullated rats, where the hypoglycaemia was only maintained for about an hour after the alloxan injection, the hyperglycaemic response may perhaps be regarded as not inhibited but only delayed It seems possible that in the absence of adrenaline secretion there is no rapid mobilization of liver glycogen, but that the release of glucose occurs slowly, perhaps under the influence of adrenal cortical hormones, so that the normal blood sugar level is restored and hyperglycaemia is produced later

The hypoglycaemic response is presumably caused by release of insulin from the islet cells There is evidence for this in the response of the rats that were already diabetic and in the difference *between the responses of the normal rats that did, and did not,* develop diabetes after the test injection of alloxan The very great rise in the blood sugar level occurring in the alloxan diabetic rats is an example of a situation the reverse of that considered in the last paragraph here the hyperglycaemia is elicited in the absence of all hypoglycaemic response In the response of the normal rats to alloxan, those that eventually became diabetic [illegible] or at least no rise in the

rise This suggests that there was a greater release of insulin in the first group, as would be expected if alloxan diabetes like that produced by hypophyseal extracts or glucose, is considered to be caused by overstimulation of the islet cells Naturally, the stimulation producing diabetes would be greater than that which did not

Some relation might be assumed between the initial hypoglycaemia and the same effect observed a few hours later It might be that alloxan causes a profound hypoglycaemia starting immediately and lasting several hours, on which is superimposed a period of hyperglycaemia However, the extra-pancreatic nature of the second phase of hypoglycaemia, already discussed in this paper, is against this assumption, and our explanation of the initial hypoglycaemia requires the presence of the pancreas

Three main arguments supporting the hypothesis that alloxan is involved in the release of insulin by the islet cells were given by us in previous papers (24, 29, 30) (1) after administration of

glucose, alloxan is present in the blood (33), (ii) in the presence of alloxan inhibitors (BAL, nicotinic acid), the hyperglycaemia induced by glucose is increased. This can be ascribed to a lack of insulin secretion by the pancreas, because the effect is not observed in alloxan diabetic and eviscerated rats, whether these are treated with insulin or not, (iii) intravenous administration of a massive amount of glucose causes islet cell damage, as is shown by an impaired tolerance to sugar twenty-four hours later. This can be prevented by giving BAL at the same time as the glucose

Several objections may be raised against these arguments The presence of alloxan in the blood after administration of glucose, as has been reported by Schuoler (33), could not be confirmed by us (23) Alternative explanations for the effect of BAL and nicotinic acid are possible. We therefore welcome the additional argument which we believe is provided by the results [illegible] described in this paper

[illegible]

recognizable activity of the islet cells. The majority of investigators have reported only degenerative lesions, but on the whole these were observed several hours after the administration of alloxan. Islet cell division or hypertrophy of the nucleus are mentioned by several authors (3, 6, 14, 15, 20). If the islet cells are injured by treatment with pituitary extracts or alloxan, a subsequent treatment with alloxan may stimulate regeneration (8, 11) The degranulation of the beta-cells following administration of alloxan, which is usually regarded as a sign of degeneration, may equally well be a sign of a massive release of insulin

If it is supposed for a moment that administration of carbohydrates causes the release of alloxan somewhere in the body and this acts as the stimulus for the secretion of insulin, it is difficult to see why the resulting hypoglycaemia is not accompanied by a hyperglycaemic effect like that observed after treatment with [illegible]

for the beta-cells

It is possible that the release of insulin involves the oxidation of a precursor possessing sulphydryl groups to another substance *with disulphide bridges, this last being perhaps insulin itself* It is generally known that alloxan and related substances can act as oxidizing agents in this way This hypothesis may perhaps accord with the views of Lazarow, who has emphasized several times the importance of sulphydryl groups to the function of the islet cells (31)

SUMMARY

The blood sugar levels 30, 60 and 150 minutes after the intravenous administration of alloxan were studied in normal, adrenalectomized, adrenal demedullated, and alloxan diabetic rats The main results were

1 An initial slight fall and delayed rise of the blood sugar level in normal animals which eventually became diabetic, in contrast with the immediate rise in those which did not develop diabetes,
2 A sharp fall to hypoglycaemic levels within thirty minutes in animals adrenalectomized four days previously,
3 *A fall followed by a rise in demedullated animals*,
4 A sharp rise, reaching a peak after one hour, in diabetic animals

The conclusions drawn from these and other data were that alloxan causes an initial hypoglycaemia which is masked by a hyperglycaemia if the liver can respond with a sufficient output of glucose This hypoglycaemia is independent of the one that is usually observed five to ten hours after administration of alloxan

The possible importance of alloxan or related substances in the release of insulin by the pancreatic islets is discussed in connection with these observations

ACKNOWLEDGEMENTS

The authors are indebted to Drs Louwerens and de Wied for many valuable suggestions and assistance during the experiments, to Miss Hut and Miss de Jonge for technical assistance, and to Mr Williams and Dr Rumke for a careful revision of the manuscript

REFERENCES

1 BAILEY, C C, *Proc Soc exp Biol*, *N Y*, 71, 580 (1949)
2 BAILEY, C C and BAILEY, O T, *J Amer med Ass*, 122, 1165 (1943)
3 BAILEY, C C, BAILEY, O T and LEECH R S, *Bull New Engl med Cent*, 7, 59 (1945)
4 BANERJEE, S, *J biol Chem*, 158, 547 (1945)
5 BANERJEE, S and BHATTACHARYA, G, *J biol Chem*, 175, 923 (1948)
6 BERTHOUD, E, *Pr méd*, 54, 875 (1946)
7 BHATTACHARYA, G, *J biol Chem*, 182, 739 (1950)
8 CAPELLATO, M, *Boll Soc ital Biol sper*, 25 1060 (1950), cited in *Excerpta med*, *Amst*, III, 5, 13 (1951)
9 CARRASCO-FORMIGUERA, R, *Arch Biol Pat*, 1, 107 (1948)
10 CORKILL, A B, FANTI, P and NELSON, J F, *Med J Aust*, 31 285 (1944)
11 DESCLAUX, P SOULAIRAC A and BROCHERIOU, J, *C R Soc Biol*, *Paris*, 142, 944 (1948)
12 DUFF, G L *Amer J med Sci*, 210 381 (1945)
13 DUNN, J S, *Lancet*, 1, 664 (1943)
14 DUNN, J S, DUFFY, E, GILM, M D, KIRKPATRICK, J and MCLETCHIE, N G B, *J Physiol*, 103 233 (1944)
15 FALLER, A and ARNOLD, A, *Bull Histol Tech micr*, 25 194 (1948)
16 [illegible]
17 [illegible]
18 [illegible] 89 (1944)
19 GOLDNER, M G and GOMORI, G, *Proc Soc exp Biol*, *N Y*, 65, 18 (1947)
20 GOMORI, G and GOLDNER, M G, *Proc Soc exp Biol*, *N Y*, 54, 287 (1943)
21 HOUSSAY B A, ORIAS, O and SARA, I, *Science* 102, 197 (1945)
22 HUGHES, H, WARE, L L and YOUNG, F G, *Lancet*, 1, 148 (1944)
23 HUISMAN, T H J, VAN HULTEN, A and STEL, H *Biochim Biophys Acta*, 6, 290 (1950)
24 HUISMAN, T H J, LAMMERS, W and SIDERIUS, P, *Acta* [illegible]
25 [illegible]
26 [illegible] 1937)
27 [illegible] *c Exp Biol*, N Y, 57, 143 (1944)

28 Kirschbaum, A, Wells, L J and Molander, D, *Proc Soc exp Biol, N Y*, 58, 294 (1945)
29 Lammers, W and Gaarenstroom, J H, *Abstr XVIII int physiol Congr*, p 323 (1950)
30 Lammers, W, Siderius, P and Gaarenstroom, J H, *Acta physiol pharm néerl*, 1, 193 (1950)
31 Lazarow, A, *Physiol Rev*, 29, 48 (1949)
32 Lukens, F D W, *Physiol Rev*, 28, 304 (1948)
33 Schiøler, P, *Biochim biophys Acta* 2, 260 (1948)
34 Shipley, E G and Beyer, K H, *Endocrinology*, 40, 154 (1947)
35 Wrenshall, G A, Collins-Williams, J and Best, C H, *Amer J Physiol* 160, 228 (1950)

DISCUSSION

Lukens I should like to have Professor Gaarenstroom's comments on the results of one of our experiments Dogs were perfused with 5% glucose solution for twenty days into the common hepatic artery After eighteen days, hypoglycaemia (30–40 mg per cent in the venous blood) developed, but the dogs were still active and eating well Thus was a very striking [illegible] the portal vein did not alter the [illegible] *crinology*, in the press) [illegible] such an infusion of glucose?

Gaarenstroom Alloxan may perhaps originate in the pancreas If glucose is infused into the pancreatic artery, it affects the pancreas immediately Insulin, perhaps by the mediating action of alloxan, will then be secreted faster and in greater quantities and thus give rise to hypoglycaemia If glucose is infused into the portal vein, on the other hand, it will be converted to glycogen in the liver, no hypo- [illegible] will be secreted [illegible] xan via the [illegible]

Gaarenstroom There is an initial hypoglycaemia which is very mild If alloxan is injected, it produces a hyperglycaemic response which may mask this hypoglycaemia

Lazarow In your early studies, when glucose and BAL were used, what diet was fed to the animals? We have carried out similar experiments using glucose and cysteine but we did not find that a large dose of glucose, given twenty-four hours beforehand, affected the glucose tolerance test significantly Perhaps this difference may be due to differences in the diet used

GAARENSTROOM We used a Government diet, it contained about seventy per cent carbohydrate and twenty per cent protein BAL was given two hours before and then again just before the glucose

LAZAROW Did you investigate the effect of giving cysteine or nicotinic acid?

GAARENSTROOM No

LAZAROW [illegible]
indirect prote [illegible]
the alloxan, [illegible]
more effective than nicotinic acid One possible explanation of the protective effects of these compounds would be that they are both excreted as *N* methyl derivatives Since the methyl groups would come from methionine, sulphydryl groups would be liberated, presumably as homocysteine

LUKENS How were the nicotinic acid derivatives administered?

LAZAROW They were given parenterally They may therefore have caused some adrenaline to be released If the effect were due to the release of adrenaline, would you expect to get better protection after a one-hour latent period?

LUKENS Yes

LAZAROW If the protection were due to the release of adrenaline, I would expect nicotinic acid to be a better protective agent, for this substance produces a much more pronounced vascular effect than does nicotinamide On the other hand, we have found that nicotinamide protects more effectively than does nicotinic acid This fact seems to correlate well with the observation that nicotinamide is excreted as an *N*-methyl derivative to a much greater extent than is nicotinic acid

CONN It may be of some interest in connection with Professor Gaarenstroom's earlier work, in which pretreatment with BAL intensified alimentary hyperglycaemia, that administration of reduced glutathione intravenously to a normal man resulted in rather marked glycosuria which began more than four hours after the glutathione had been administered This man had been excreting 1 1–1 2 g of glucose per day for a considerable time on a metabolic balance study (This is the normal rate of urinary glucose loss) On one day he was given 4 g of reduced glutathione intravenously For the next four hours there was no change in renal excretion of glucose However, during the following sixteen hours he excreted more than 5 g of glucose By the next day the glucose content of the urine had returned to base-line values This is in contrast to our finding that, when reduced glutathione is given in man *during the development of ACTH diabetes*, a transient decrease in severity of the glycosuria and hyper-

urinary uric acid nor urinary nitrogen increased in amount Also the delayed onset of glycosuria after the administration of glutathione

the glycosuria or hyperglycaemia in alloxan diabetic rats

CONN The experiment was done four times in the same subject and always gave the same response I should add that we always get a very marked diabetes when ACTH is given to this subject. It is for this reason that he was chosen for these experiments

WILHELMI What was the food intake in this experiment?

CONN Meals were given at 8 a m noon and 5 p m, the intake of carbohydrate was 300 g per day, of protein 100 g and of energy 3400 kg cal The carbohydrate was equally distributed between the meals, though the protein was not The upper part of the tolerance curve indicated that it was not an effect due to response via the adrenal

to insulin secretion, I would like to describe the following experiments on dogs which we recently carried out We took one group of normal dogs and another group of dogs rendered permanently diabetic by previous injection of alloxan Both groups received 5 mg of alloxan per kg body weight This was injected in small volume at low pressure and under anaesthesia, into Wirsung's duct ligatured on the duodenal side, that is, the injection was made into the excretory duct Following the injection, there was a fall of about thirty per cent in the blood sugar level in ten minutes in the normal animals, with a return to normal within thirty minutes. However, in the dogs with permanent meta-alloxanic diabetes and severe hyperglycaemia (300–400 mg per cent), the same injection of alloxan gave rise to a discrete hypoglycaemia of ten to twelve per cent which was reduced

after ten minutes We conclude from these experiments that in normal

[illegible]

of its derivatives, which acts as the stimulant

To continue with my account of these experiments—the injection of physiological saline, even in small volume and at very low pressure, may produce a slight hypoglycaemia Dilatation of the excretory ducts seems to be the cause It is conceivable, therefore, that during the course of digestion, and to compensate the hyperglycaemia resulting from a mixed meal, a part of the insulin secreted is produced by simple distention of the excretory ducts of the pancreas when it discharges into the duodenum

CANDELA I would like to ask Professor Loubatières whether he examined the pancreas histologically following the injection of alloxan, and if he observed any signs of inflammation

LOUBATIÈRES Some sections are being prepared at the moment, but I do not yet know the results

BEST I would like to ask Professor Loubatières why he gave alloxan via the pancreatic duct This method may lead to interesting results, but I know he will agree that more controls are needed to make sure that the effect is due to the alloxan For example, one might test histamine and other chemicals to see whether they have any effect on insulin liberation when given in this manner Is there an increased flow of blood to the pancreas?

LOUBATIÈRES All this is perhaps a little premature It is probably a derivative of alloxan which acts There are valid arguments in favour of this supposition which are presented in my own paper It [illegible]

[illegible] of the dilatation of the ducts from the actual effect of alloxan on the blood sugar level The duodenal end of Wirsung's duct was ligatured and a cannula placed in the glandular end Physiological saline was perfused through this cannula at a pressure of 1 cm of water (This is possible since the liquid escapes through the accessory excretory ducts, fluid is, in fact, found in the duodenum) This perfusion sometimes produced a slight but definite fall in the blood sugar level It is therefore possible that the hypoglycaemia produced by injection of a solution of alloxan into Wirsung's duct resulted from two superposed effects dilatation of the ducts, and stimulation of the islets of Langerhans by alloxan

LUKENS There is a remarkable case on record of a patient with hypoglycaemia on whom an operation was performed and a cyst removed from the tail of the pancreas There were no beta-cells but the hypoglycaemia was relieved It makes one wonder how many stimuli there are to the islets!

DIABETES AND BIOLOGICAL OXIDATION OF URIC ACID

By

MERVYN GRIFFITHS

Australian Institute of Anatomy,
Commonwealth Health Department, Canberra, Australia

INTRODUCTION

The discovery by Dunn, Sheehan and McLetchie that diabetes mellitus follows injection of alloxan (4) was the stimulus for active enquiry into the chemical and biological properties of alloxan Particularly noteworthy were the observations that

logues mentioned each have a triketo grouping ... cules, and this, presumably, is responsible for the ... GSH It would appear, since sulphydryl comp... the diabetogenic effect of alloxan, that a decrease ... concentration is essential for the appearance of di... an intact configuration -NH-CO-NH- is equally ...

DIABETOGENIC ACTION OF URIC ACID

It should be emphasized that the carbodiimide ... the only combination of chemical groups capable of ... islet cells, since Patterson has shown that dehyd... is diabetogenic (17) However, the evidence ... time suggested that uric acid might be diabetog... of its configuration at positions 1, 2 and 3 if it were ... animals depleted of sulphydryl groups This ... rabbits whose ...

oped hyperglycaemia and glycosuria lasting some ...

one rabbit persistent diabetes was found Collins-Williams and Bailey and Loubatières *et al* have not been able to confirm these findings unequivocally (3, 14) However, Liguori in Cagliari found that injection of uric acid into rats deficient in *gruppi solforati* brought about a transient diabetes (13)

In spite of the agreement between our results and Liguori's there are points of difference For example, Liguori found it necessary to feed his rats on the deficient diet for only fifteen days before injecting uric acid With our rabbits it was necessary to feed a sulphydryl deficient diet for five to six weeks before the blood GSH level had been lowered enough for uric acid to exert a diabetogenic effect Liguori did not determine the blood GSH concentration in his rats In my experience, rats are completely resistant to repeated large doses of uric acid Grunert and Phillips later found the same thing (7), but unfortunately the dose of uric acid which they used was very small

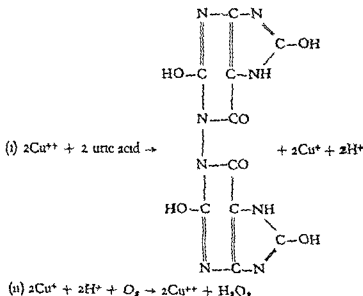
Xanthine and uracil have no diabetogenic effect in rabbits which have a low blood GSH concentration The negative result in the case of uracil indicates that the diabetogenic effect of uric acid is not simply a property of the configuration at positions 1, 2 and 3 together with a deficiency of sulphydryl groups At the ... no apparent reason for the negative result with ... ow known, ... the tissues

ENZYMIC OXIDATION OF URIC ACID

When the diabetogenic activity of uric acid had been established, an investigation was made of the biochemical properties of uric acid to try and find out why it is diabetogenic It was observed that ferricytochrome *c* is reduced by uric acid at neutral pH (15) The rate of reduction increases with increase of pH Thus, the intense absorption bands of ferrocytochrome *c* can be seen in the reversion spectroscope when a little uric acid is added to a solution of ferricytochrome *c* in phosphate buffer at pH 9 0 Since the reduction of ferricytochrome *c* involves the transfer of an electron from the uric acid to cytochrome, resulting in the reduction of Fe^{+++} to Fe^{++}, a proton leaves the uric acid, resulting in dehydrogenation. The oxidation product

may then be termed dehydrouric acid, analogous with dehydroascorbic acid or cystine (which are the products of heavy metal catalysed oxidation of ascorbic acid and cysteine respectively) Unfortunately the strong absorption of cytochrome *c* in ultraviolet light interferes with the spectrophotometric measurement of the oxidation of uric acid, since the absorption maxima of uric acid occur at ultraviolet wavelengths However, copper has been found to be a suitable catalyst at high pH values with molecular oxygen as the hydrogen acceptor A detailed account of this work is in the press so it will be sufficient here to describe briefly the characteristics of the oxidation

By use of the conventional Warburg manometric technique, a rapid uptake of oxygen can be demonstrated with a solution of uric acid in phosphate buffer at pH 11 8 in the presence of catalytic amounts of cupric chloride At pH 8 2 the oxidation is scarcely perceptible, but it increases in rate with increase in pH until at pH 12 4 it is quite rapid One gram-atom of oxygen is taken up for each molecule of uric acid oxidized. The reaction might then be formulated



The dehydrouric acid decomposes and is then decarboxylated This is strong evidence that some allantoin is formed (cf 8) The activation energy of the system uric acid—copper—O_2 is relatively low (about 16 950 cal/mole) This is in agreement with the postulate by Michaelis that the formation of a relatively stable *addition compound, within which intramolecular transfer* of electrons can occur, overcomes the high activation energy involved in the reduction of molecular oxygen (16)

example, the extinction at 293 mμ which is the absorption maximum of uric acid at high pH, decreases rapidly at room temperature *in the presence of copper or of uricase In addition, the* increases in extinction which Praetorius observed at wavelengths of 310–340 mμ when uric acid is oxidized in the presence of uricase (18), also occur when copper is the catalyst Praetorius also showed that the formation of the substance responsible for the increase in extinction at these wavelengths was a result of the enzymic catalysis and not of a non-enzymic change following the catalysis It is possible then, that the increase in extinction at wavelengths of 310–340 mμ represents the absorption spectrum of dehydrouric acid at high pH

The results of these experiments would seem at first sight to *have little application to the aetiology of diabetes since the* oxidation *in vitro* has to be carried out at high pH However, it has been found that a crude cytochrome—cytochrome oxidase system prepared from ox heart muscle, together with cytochrome *c* will catalyse a considerable oxidation of uric acid at pH 7 9

The reaction may be formulated

(i) 2 ferricytochrome *c* + 2 uric acid →
2 ferrocytochrome *c* + dehydrouric acid + 2 H^+

(ii) 2H^+ + 2 ferrocytochrome *c* + $\frac{1}{2}O_2$ →
H_2O + 2 ferri-cytochrome *c*

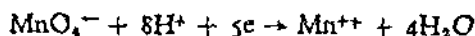
The implication of these results is that uric acid could be oxidized in an organism lacking uricase, such as man In support

of this, Buzard, Bishop and Talbott find that, in man, the recovery of isotopically labelled uric acid is much less than that expected on the basis of calculations of the size of the uric acid pool and its turnover rate They suggest that uric acid may not be the sole end-product of purine metabolism

With the assumption that uric acid can be oxidized in man, it could be argued, by analogy with the case of dehydroascorbic acid, that the dehydrouric acid formed would be diabetogenic [1] It is unlikely that dehydrouric acid decomposes to alloxan *in vivo*, since Lee and Stetten have shown that alloxan is not formed in any significant amount from isotopically labelled uric acid injected into rabbits deficient in glutathione (11)

DIABETOGENIC OXIDATION PRODUCTS OF URIC ACID

To test the possibility that dehydrouric acid is diabetogenic, an attempt was made to prepare it by stoichiometric dehydrogenation of uric acid with potassium permanganate It is well known that oxidation of uric acid by permanganate under alkaline conditions is followed by the formation of allantoin,



permanganate in the presence of excess hydrogen ions occurs according to the reaction

$$MnO_4^- + 8H^+ + 5e \rightarrow Mn^{++} + 4H_2O$$

If potassium permanganate is added drop by drop to a well-stirred suspension of uric acid in dilute sulphuric acid, the permanganate is decolourized If sufficient permanganate is added, together with more than sufficient sulphuric acid to neutralize the hydroxide ion formed, the uric acid disappears into solution It is better, however, to have an excess of uric acid rather than an excess of permanganate, since the remaining uric acid can be removed by filtration The filtrate contains a substance capable of oxidizing iodine ion to iodine Presumably this oxidizing agent is dehydrouric acid The substance decomposes within

[1] It is interesting to note here that the islets of Langerhans of fish possess a cytochrome oxidase system (10)

a matter of minutes and then no longer oxidizes iodide The Mn^{++} may be removed from this solution by passing it through a column of Amberlite IR–100(H)

Rabbits injected intravenously with the effluent adjusted to pH 4–5 became permanently diabetic The dose corresponded to the uric acid oxidized by 250–350 mg $KMnO_4$ per kg body weight Rabbits injected with the solution have remained diabetic for two months, with blood sugar values ranging from 290 to 420 mg per cent Severe hypoglycaemia accompanied by convulsions occurred in some of these animals a few hours after injection of the solution Injection of glucose abolished the convulsions and the animals survived The diabetogenic compound in the solution has been identified spectrophotometrically as alloxan[1] Thus dehydrouric acid, formed by the removal of electrons from uric acid by permanganate, decomposes to allantoin or to alloxan according to the pH

If the oxidation of uric acid is carried out in the absence of sulphuric acid, by quickly adding potassium permanganate, drop by drop, to a well-stirred suspension of uric acid until the pH rises to 7 1, a clear yellow solution is obtained when the excess uric acid is removed on a fast filter paper The filtrate oxidizes iodide ion to iodine and is reasonably stable at neutral pH On acidification with sulphuric acid the yellow colour

perties

DIABETOGENIC ACTION OF ASCORBIC ACID

It will be recalled that the diet deficient in methionine and cystine used in the work on uric acid diabetes contained a considerable amount of ascorbic acid (6) It is possible that a synergism exists between ascorbic acid and uric acid in the induction of diabetes, since *both compounds can be dehydrogenated by*

[1] This identification was made possible by the observation that the ultraviolet absorption spectrum of alloxan at pH 2 1 shows a maximum at 2100 A. The instrument used was a Hilger quartz photoelectric spectrophotometer

the cytochrome–cytochrome oxidase system To test this possibility, ascorbic acid was injected into rabbits whose blood GSH level had been lowered by feeding the diet deficient in methionine and cystine. Rabbits given intraperitoneal injections of 1 g per kg body weight died within twenty-four hours With a dose of 400 mg per kg injected intravenously the animals survived and a few days later were injected with the same dose again. The animals survived but failed to develop diabetes A diabetogenic effect of ascorbic acid cannot be entirely discounted by this experiment Possibly ascorbic acid is diabetogenic in sulphydryl deficient rats

REFERENCES

1 BRUCKMANN, G and WERTHEIMER, E, *J biol Chem*, 168, 241 (1947)
2 BUZARD, J, BISHOP, C and TALBOTT, J H, *J biol Chem*, 196, 179 (1952)
3 COLLINS-WILLIAMS, J and BAILEY, C. C, *Proc Soc exp Biol*, *N Y*, 71 583 (1949)
4 DUNN, J S, SHEEHAN, H L and MCLETCHIE, N G B, *Lancet*, 1, 484 (1943)
5 GRIFFITHS, M, *J biol Chem*, 172, 853 (1948)
6 GRIFFITHS, M, *J biol Chem*, 184, 289 (1950)
7 GRUNERT, R R and PHILLIPS, P H, *Proc Soc exp Biol*, *N Y*, 76, 642 (1951)
8 KLEMPERER, F W, *J biol Chem*, 160, 111 (1945)
9 LAZAROW, A, *Proc Soc exp Biol*, *N Y*, 61, 441 (1946)
10 LAZAROW, A and COOPERSTEIN, S J, *Biol Bull*, *Wood's Hole*, 100, 191 (1951)
11 LEE, J M and STETTEN, D, Jr, *J biol Chem*, 197, 205 (1952)
12 LEECH, R M and BAILEY, C C, *J biol Chem*, 157, 525 (1945)
13 LIGUORI, G, *Rass med Sarda* 1, 1, (1951)
14 LOUBATIÈRES, A, BOUYARD, P and DE LACLOS, F, *Arch Sci Physiol.*, 6, 53 (1952)
15 MARGULES, L and GRIFFITHS, M, *Arch Biochem*, 29 225 (1950)
16 MICHAELIS L, in *Currents in Biochemical Research*, ed GREEN, D E., New York Interscience Publishers, Inc (1946)
17 PATTERSON, J W, *J biol Chem*, 183, 81 (1950)
18 PRAETORIUS, E, *Biochim biophys. Acta*, 2, 602 (1948)
19 WILLIAMS, J N, Jr, DENTON, A E and ELVEHJEM, C. A., *Proc Soc exp Biol*, *N Y*, 72, 386 (1949)

DISCUSSION

YOUNG Am I correct in believing that dehydrouric acid has not yet been positively characterized but merely identified spectrographically?

[illegible]

GRIFFITHS Even the primary oxidation product of glucose was not known until recently!

YOUNG Do you definitely believe that dehydrouric acid is formed in the body from uric acid by the action of uricase?

GRIFFITHS Yes I do

LUKENS If this substance is formed in the body—and we should note that you produce it at an unphysiological pH and with an unnatural oxidizing agent—it must be by an enzyme system What happens to uric acid on treatment with uricase?

GRIFFITHS I believe the primary oxidation product must be dehydrouric acid Uricase may perhaps have a metal prosthetic group [illegible]

GRIFFITHS Yes I do

YOUNG Uricase and cytochrome oxidase may then both be concerned with the production of dehydrouric acid in the body, according to your views

CONN What was the condition of the adrenals in the animals fed on the diet deficient in cystine and methionine?

GRIFFITHS The adrenals were rather brown and depleted in lipoid but we did not examine them histologically

BEST What was the composition of this deficient diet?

GRIFFITHS We used a peanut-meal diet

LONG Did the rabbits not lose a great deal of weight when on the deficient diet?

GRIFFITHS Yes, their weight fell from about 800 g to about 600 g, but they were still lively, as we used strong wild animals straight from the bush

WILHELMI Methionine and cystine may be needed for the synthesis of growth hormone in the pituitary

LONG If you take another group of rabbits with an adequate amount of cystine and methionine in the diet do you still observe this effect of uric acid

GRIFFITHS If methionine is added to the diet there is a protective

measured

BEST Was there any fall in the concentration of glutathione in the blood of the animals on the diet deficient in sulphur or on the reduced caloric intake?

GRIFFITHS With the diet low in sulphur the blood glutathione level falls with time I do not know what happens if the caloric intake is restricted

LONG If glucose is given do you think there will be an abnormal tolerance curve?

GRIFFITHS Yes I think so I might also add that the amount of insulin extractable from the pancreas was about one-third of normal before the uric acid injection

probably thought that it was unnecessary to add ascorbic acid to the diet because he was working with rats which synthesize their requirements for themselves

LOUBATIÈRES There are three points in particular to which I would like to draw your attention Mr Griffiths

(i) Although diabetes provoked by uric acid may be an experi

four days after injection of uric acid reveals uric acid encysted in an epiploic sheath Given this situation one may wonder whether the uric acid itself is responsible for the diabetes or whether one or several of its chemical derivatives which are slowly reabsorbed may be the cause

(iii) The physiological interest of this research despite its difficulty should be emphasized French clinicians in the nineteenth century included under the heading of arthritic diathesis diabetes mellitus on

the one hand and various arthritic manifestations, especially gout, on the other It is therefore possible that this research on experimental diabetes corresponds with a clinical reality

GRIFFITHS To take Professor Loubatières' first two points in order (i) I should emphasize that these were not normal animals, but they were quite lively, (ii) uric acid was observed in the peritoneum and this would suggest that large amounts were not absorbed I agree that uric acid itself may not be diabetogenic. Uric acid was, however, found to be very rapidly absorbed in the experiments of Collins-Williams and Bailey However, there is one puzzling point, in one of their experiments there was a very great hyperglycaemia but no increase in the amount of uric acid in the blood

LAWRENCE There was a condition in England a hundred years ago called 'gouty glycosuria' which has now completely disappeared This was probably related to the obese nutritional state It is possible, ... cosuria but that

dence
idence
idence

of diabetes in the gouty patients themselves than in the general population

LOUBATIÈRES I should like to return to the physiopathological viewpoint and to the arthritic diathesis of the old clinicians Dr Lawrence probably misunderstood my remarks, it is not a question of the simultaneous presence of gout and glycosuria in certain patients Arthritic diathesis, considered as a nutritional disorder, tends either towards diabetes mellitus or towards gout It is a matter of lack of ... on or the other

thritic diathesis' include poly-
oints?

LAZAROW You have reported that one rabbit developed permanent diabetes which persisted for several months (GRIFFITHS, M, *J biol Chem*, 184, 289, 1950) Have you been able to produce permanent ... more animals? Your results would be more convincing

attempts using continued injection
which uricase is present in tissues
itable Perhaps monkeys or birds

could be used

YOUNG Have you studied these phenomena in the Dalmatian Coach-hound and compared them with those occurring in other types

of dog in the way that Dr Conn compared the responses towards the diabetogenic action of ACTH in Dalmatian and mongrel dogs?

GRIFFITHS The Dalamatian Coach hound has uricase and therefore probably would be of no use

CONN There is a somewhat greater diabetogenic response to ACTH in the Dalmatian Coach hound than in mongrel dogs but very large

[illegible]

account for the inconsistency of the response One should therefore add a control experiment to determine the constancy of the diabetes produced by alloxan for example

GRIFFITHS This was done by Houssay he gave alloxan to rats on a diet deficient in cystine and methionine and found that the response to alloxan was enhanced

LAZAROW How pure was the sample of uric acid which you used? Is it possible that there may have been sufficient alloxan present in the uric acid sample to cause diabetes?

GRIFFITHS I do not think so

LAZAROW The doses of uric acid used in your experiment were 1–2 g per kg The presence of a small amount of alloxan as a contaminant might therefore provide a diabetogenic dose of this latter compound

GRIFFITHS There is no reason to suspect that alloxan was present

BEST How physiological is the condition of the animals given large doses of alloxan *itself* immediately after the treatment?

[illegible]

If t [illegible]

all[illegible]

may not have been more unphysiological than many others in which very large doses of alloxan itself were given

LONG It would be interesting to know whether the continued administration of small doses of alloxan would give rise to diabetes

LAZAROW We gave repeated subthreshold doses of alloxan to rats Some animals developed diabetes after a few injections others became

the one hand and various arthritic manifestations, especially gout, on *the other It is therefore possible that this research* on experimental diabetes corresponds with a clinical reality

GRIFFITHS To take Professor Loubatieres' first two points in order (i) I should emphasize that these were not normal animals, but they were quite lively, (ii) uric acid was observed in the peritoneum and this would suggest that large amounts were not absorbed. I agree that uric acid itself may not be diabetogenic Uric acid was, however, *found to be very rapidly absorbed in the experiments of Collins-*Williams and Bailey However, there is one puzzling point, in one of their experiments there was a very great hyperglycaemia but *no* increase in the amount of uric acid in the blood

LAWRENCE There was a condition in England a hundred years ago called 'gouty glycosuria' which has now completely disappeared This was probably related to the obese nutritional state It is possible, [illegible] rise to *glycosuria but that*

[illegible] dence of diabetes in gouty families It was found that there is a high incidence of diabetes in the families of gouty subjects but no greater incidence of diabetes in the gouty patients themselves than in the general population

LOUBATIÈRES I should like to return to the physiopathological viewpoint and to the arthritic diathesis of the old clinicians Dr Lawrence probably misunderstood my remarks, *it is not a question of* the simultaneous presence of gout and glycosuria in certain patients Arthritic diathesis, considered as a nutritional disorder, tends either towards diabetes mellitus or towards gout It is a matter of lack of compensation in one direction or the other

LAWRENCE Then did the term 'arthritic diathesis' include polyarthritis and all other disorders of the joints?

LOUBATIÈRES I believe so

LAZAROW You have reported that one rabbit developed permanent diabetes which persisted for several months (GRIFFITHS, M, *J biol. Chem*, 184 289, 1950) Have you been able to produce permanent diabetes in any more animals? Your results would be more convincing if this could be done

GRIFFITHS We have made some attempts using continued injection of uric acid, but I think animals in which uricase is present in tissues such as the liver would not be suitable *Perhaps monkeys or birds could be used*

YOUNG Have you studied these phenomena in the Dalmatian Coach-hound and compared them *with those occurring* in other types

DOES ALLOXAN PLAY A PART IN THE PATHOGENESIS OF DIABETES MELLITUS?

By

A LOUBATIÈRES

Laboratoire de Physiologie Appliquée, Institut de Biologie, Montpellier, France

INTRODUCTION

The biological role of alloxan and the part played by this

of insulin and phlorrhizin and also of removal of the adrenals and of the hypophysis on this hyperalloxanaemia

Such investigations are, however, of value only when the procedures employed are described in adequate detail The first part of our paper is devoted to this aspect of the work

ESTIMATION OF ALLOXAN

Principle

The method used is a variation of that described by Archibald, under No VI in his paper (1) It is based on the fluorimetric estimation of the alloxazine formed when alloxan reacts with *ortho*-phenylenediamine

Reagents

(a) Anticoagulants neutral sodium fluoride (R P) in 4 per cent solution, heparin (Hoffman-La Roche or Choay)

(b) Substances used for the deproteination of blood pure crystalline neutral sodium sulphate (R P) ($Na_2SO_4,10H_2O$) in

diabetic only after forty or more injections It is important to note that the quantitative difference between a subdiabetogenic and a

LONG The effect of continuous intravenous infusion should be studied

LUKENS In some clinical experiments, up to 600 mg of alloxan per kg were given over a period of 1—1½ hours It appears that there was a very rapid inactivation of the alloxan, because there was no effect at all Houssay notes that the speed of injection is very important

LONG It would be interesting to know whether there are physiological situations in which a rise in concentration of alloxan of this order is found

YOUNG It is possible that a local concentration is important

LAZAROW In estimating the dose of alloxan required to produce diabetes, it must be remembered that its half-life at pH 7 4 and 37°C is about one minute Therefore the amount of alloxan involved in the destruction of the beta-cells is probably a very small fraction of the injected dose (40 mg per kg)

BEST Was any alloxan given in your infusion experiments Dr Lukens?

LUKENS. No

VERZÁR Must we regard alloxan as a product normally associated with the metabolism of carbohydrate?

GRIFFITHS I believe alloxan is a product of uric acid oxidation but not of carbohydrate oxidation

The total time taken for these operations must not exceed 3–4 minutes as the substance to be estimated is very unstable

Fluorimetric apparatus

T[illegible]
imp[illegible]
and[illegible]

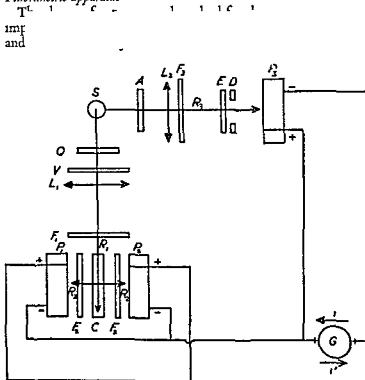


FIG 1

Diagram of fluorimeter after Petit (ref 15)

rec[illegible]
ga[illegible]
R_1 [illegible]
P_3 [illegible] current generated by P_3

[illegible]

comparative study

The fluorimeter (Fig 1) is provided with an optical compensa-

3 per cent solution, pure crystalline sodium tungstate (R P) ($Na_2WO_3,2H_2O$) in 10 per cent solution, pure *anhydrous* sulphuric acid from which normal, 0 75 N and 0 002 N solutions are prepared

(c) Reagents used in the estimation itself pure crystalline *ortho*-phenylenediamine (Kahlbaum or Eastman-Kodak), kept in the dark, pure glycerol (R P), sp gr 1 262, pure crystalline sodium phosphate (Billault) (NaH_2PO_4,H_2O), in 13 8 per cent (w/v) solution

Stock solution of *ortho*-phenylenediamine 50 mg in 100 ml. glycerol (this solution keeps for several months in the dark), the working solution is prepared by diluting 1 ml of this stock solution of *ortho*-phenylenediamine with 10 ml of phosphate buffer solution (to be used within one hour)

Alloxan (monohydrate) (synthesized by Rhône-Poulenc Laboratories under the number 3127 R P) stock solution, 1 mg/ml in 0 002 N-H_2SO_4 (renewed weekly and stored in the dark in the refrigerator), working solution, 10 µg/ml in 0 002 N-H_2SO_4 *(to be prepared before each estimation)*

Technical details

Withdraw as rapidly as possible (5 seconds) 4 ml of blood, using a syringe previously treated with heparin or fluoride, and quickly place it in a receptacle which has been similarly treated With a pipette draw off 3 ml of this blood and run it rapidly into a centrifuge tube containing 27 ml of the deproteinizing reagent (24 ml of 3 per cent Na_2SO_4, *1 5 ml of 10 per cent* Na_2WO_3 1 5 ml of 0 75 N-H_2SO_4) prepared immediately prior to the withdrawal of the blood Shake the mixture and centrifuge for 30 seconds

During the centrifugation add to each of three test tubes, *S*, *X* and *T*, 1 ml of the working solution of *ortho*-phenylenediamine, prepared immediately beforehand In addition, add to tube *S* an excess quantity of the working solution of alloxan comparable in amount to that presumed to be in 1 ml of blood (1, 2 or 4 µg) and to tube *T*, 10 ml of the deproteinizing solution Then add to tubes *S* and *X*, 10 ml of the supernatant remaining after deproteination of the blood The three tubes *S*, *X* and *T* are then placed in the dark for one hour at room temperature (18°C)

estimated are compared with the fluorescence of a control solution, which, in our experiments, is a mixture of the deproteinizing reagent and *ortho*-phenylenediamine, this choice appeared to us to be the logical one as a result of the tests we had carried out

Justification of the method

(a) *Riboflavin* The apparatus will estimate 0 005 µg /ml of riboflavin in sulphuric acid solution and detect fluorescence below this limit Moreover, there is a linear relationship between concentration of the solution and galvanometer reading

(b) *Alloxan* The fluorescence produced by a mixture of alloxan and *ortho*-phenylenediamine is less than that of riboflavin at the same concentration. Good accuracy is achieved with a solution of 0 05 µg alloxan in 0 002 N-H_2SO_4 per ml of liquid present in the cell, but lower concentrations are detectable and may be estimated to a sufficient degree of approximation. The accuracy is obviously greater the higher the concentration The same accuracy is obtainable if known quantities of alloxan are added to the deproteinizing reagent Estimations are equally satisfactory, although in our opinion less accurate, if known quantities of alloxan are added to the supernatant remaining from the deproteination of normal blood

In certain cases, though they are fortunately rare, the reaction of alloxan with *ortho*-phenylenediamine is inhibited The readings are then incorrect and have to be discarded This phenomenon is probably due to the action of fluorescence inhibitors

The accompanying graph (Fig 2) gives the results of estimations made by the internal standardization method when known quantities of alloxan were added to various reagents in the presence of *ortho*-phenylenediamine

Specificity of the method

The specificity of the method is supported by the following investigations

supernatant was concentrated threefold and mixed with *ortho*-phenylenediamine With Bagneaux blue as primary filter and Chance OY18 as secondary filter, the resultant mixture gave a fluorescence band in the region 5150–6430 A This is very close

tion device which gives maximum sensitivity and enables us to obtain a linear relationship between the concentration of the fluorescent substance in the solution and the reading taken on the apparatus This same device also compensates for current and voltage variations which may affect the intensity of the light source [illegible]

amp /mr

to that c

adjustments of the spot to the zero mark The essential points in our results were checked using a direct reading galvanometer more sensitive than the above (Sefram, 3×10^{-9} amp /mm.) This was inserted in the circuit of the two photoelectric cells placed on each side of the cell containing the fluorescent solution This latter method is, however, more open to criticism. At the beginning of our investigations we used as primary filters, blue Bagneaux filters and as secondary filters, Chance OY18 Later we used Corning 5113 as primaries and Corning 3385 as secondaries These latter appeared to be more satisfactory

Measurement of fluorescence

The reading must always be made when the ultraviolet lamp has reached its maximum intensity (25 minutes after switching on) and the apparatus must be set in the range of maximum sensitivity This is possible with the apparatus which we used

with the most

ss of alloxan)

stimated) and

finally the control tube T

The concentration of alloxan in µg /ml of blood is given by the relation $\frac{(X-T)s}{S-X}$, where s = excess alloxan in tube S in µg , and S, X and T are the readings given by the respective solutions The three readings take only 40 seconds in all

Our method is thus based on the principle of internal standardization and consequently differs in this respect from that described by Archibald *Our method uses as a reference the rate of reaction during a given period* (1 *hour*) *of a known excess quantity of exogenous alloxan placed under the same conditions as the alloxan to be estimated* The fluorescence of the solution containing excess alloxan and that of the solution whose alloxan content is being

of *endogenous* alloxan extracted from the blood of a dog with intense meta-alloxanic diabetes also has a band at 4900–5800 A. It is worthy of note that the *fluorescence maxima for all of these three substances are identical* and situated at 5150–5200 A. Again under the same experimental conditions, an extract of the blood of a normal dog gives a fluorescence band which is discrete and of

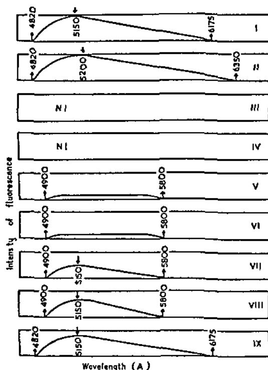


FIG 3

Comparison of fluorescence spectra given by synthetic alloxan 'endogenous' alloxan from dogs' blood and riboflavin

to that given by a solution of synthetic alloxan (10 µg/ml) in the precipitating reagent (4850–6430 A) No recordable fluorescence was obtained, however, with the deproteinizing solution alone or with the precipitate from normal (non-hyperalloxanaemic) blood

Recent experiments have shown that if Corning 5113 is used as primary filter and Corning 3385 as secondary filter under the

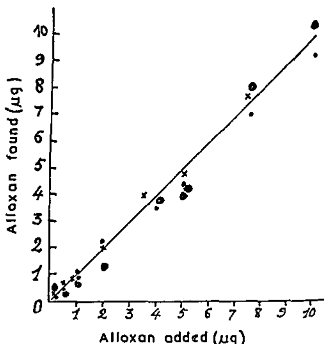


FIG 2

Estimation of alloxan by the method of internal standard zation
Alloxan dissolved in ● 0002 N-H_2SO_4 × the deproteinizing reagent ● the supernatant remaining after deproteination of the blood.

same experimental conditions, the fluorescence spectrum of a mixture of *synthetic* alloxan and *ortho*-phenylenediamine has a band in the range 4820–6175 A The fluorescence spectrum of *endogenous* alloxan, extracted from the blood of a dog subjected to fasting and rendered hyperalloxanaemic by administration of glucose, has a band at 4900–5800 A The fluorescence spectrum

same experimental conditions appeared to be less in rabbits and dogs although quantities in the range 5–6 µg /ml were commonly observed The administration of distilled water did not produce this effect

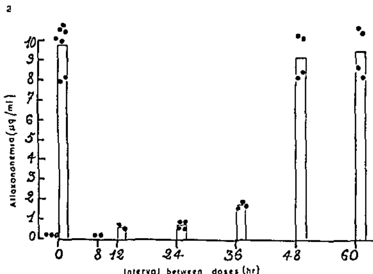


Fig 6

Effect of length of fast on height of hyperalloxanaemic wave induced by glucose (50 per cent solution 2 g per kg body wt by mouth)

First dose of glucose given at time 0 after 48 hours fast second dose given at times shown. The vertical rectangles represent the mean heights of the waves of hyperalloxanaemia observed in each group of rats

and reached its maximum intensity 45–50 minutes later, it then gradually declined and disappeared within 100 minutes Figs 5 and 7 show the effect as observed in the rat and the dog

If a rat is treated with glucose after being fasted for 48 hours, the wave of hyperalloxanaemia following a second dose of glucose will occur with the same intensity as the first only if the two doses of glucose are separated by an interval of at least 48 hours Fig 6 shows the behaviour of groups of rats treated as described in the legend It will be seen that fasting for 48 hours is

quantity of alloxan (0 2–0 3 µg /ml) which was not affected by fasting Comparable, or slightly greater, concentrations were found in rabbits, dogs and human beings (0 2–0 45 µg /ml)

2 Effect of glucose administration

Administration of a 50 per cent solution of glucose by mouth (2 g /kg body weight) to rats fasted for 48 hours resulted in the appearance of considerable quantities of alloxan in the blood

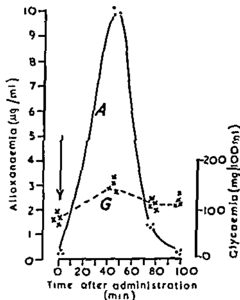


Fig 5

Effects of oral adm nistration of 50 per cent glucose solution (2 g per kg body wt) on alloxanaemia (A) and glycaem a (G) in groups of white rats previously fasted 48 hours

The maximum concentration was reached approximately forty-five minutes after ingestion of the glucose and varied between 5 and 13 µg /ml At this stage the blood sugar level was usually about 150 mg per cent Not all strains of rats showed ... of hyperalloxanaemia under the same condi- ... direction There ... which must be emphasized This effect also occurred in rabbits, dogs and human beings The degree of hyperalloxanaemia observed under the

same experimental conditions appeared to be less in rabbits and dogs although quantities in the range 5–6 µg /ml were commonly observed The administration of distilled water did not produce this effect

anc

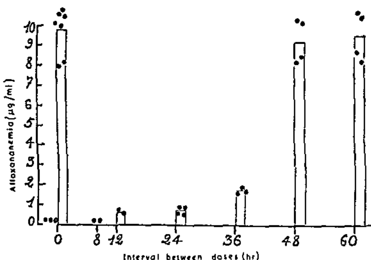


Fig 6

Effect of length of fast on height of hyperalloxanaemic wave induced by glucose (50 per cent solution 2 g per kg body wt by mouth)

First dose of glucose given at time 0 after 48 hours fast second dose given at times shown. The vertical rectangles represent the mean heights of the waves of hyperalloxanaemia observed in each group of rats.

and reached

yperalloxanaemia following a second dose of glucose will occur with the same intensity as the first only if the two doses of glucose are separated by an interval of at least 48 hours Fig 6 shows the behaviour of groups of rats treated as described in the legend It will be seen that fasting for 48 hours is

quantity of alloxan (0 2–0 3 µg /ml) which was not affected by fasting Comparable, or slightly greater, concentrations were found in rabbits, dogs and human beings (0 2–0 45 µg /ml.)

2. *Effect of glucose administration*

Administration of a 50 per cent solution of glucose by mouth (2 g /kg body weight) to rats fasted for 48 hours resulted in the appearance of considerable quantities of alloxan in the blood

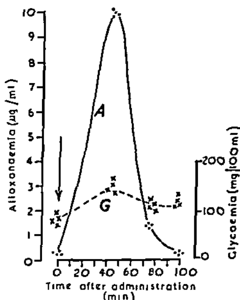


FIG 5

Effects of oral administration of 50 per cent glucose solution (2 g per kg body wt) on alloxanaemia (A) and glycaemia (G) in groups of white rats previously fasted 48 hours.

The maximum concentration was reached approximately forty-five minutes after ingestion of the glucose and varied between 5 and 13 µg /ml At this stage the blood sugar level was usually about 150 mg per cent Not all strains of rats showed such a high degree of hyperalloxanaemia under the same conditions, although the effect was always in the same direction There is, therefore, [illegible] of which must be emphasized [illegible] and human beings The [illegible] l under the

same experimental conditions appeared to be less in rabbits and dogs although quantities in the range 5–6 µg /ml were commonly observed The administration of distilled water did not produce this effect

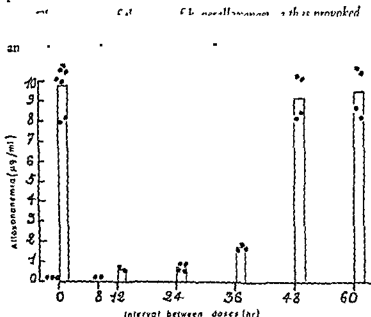


Fig 6

Effect of length of fast on height of hyperalloxanaem c u ave induced by glucose (50 per cent solut on 2 g per kg body wt by n outh)

First dose of glucose given at time 0 after 48 hours fast second dose g ven at times shown. The vertical rectangles represent the mean heights of the waves of hyperalloxanaemia observed in each group of rats.

and reached its maximum intensity 45–50 minutes later, it then gradually declined and disappeared within 100 minutes Figs 5 and 7 show the effect as observed in the rat and the dog

6 *Destruction of alloxan in the liver*

Estimations made in dogs following the provocation of hyperalloxanaemia by glucose showed that if the alloxan originated in the intestine—and alloxanaemia was more intense in the portal vein than in the mesenteric artery—then a large proportion of it must have been trapped or destroyed in the liver, since the suprahepatic alloxanaemia was less intense than that in the portal vein (maximum difference 2 5–3 µg /ml) (see Fig 7)

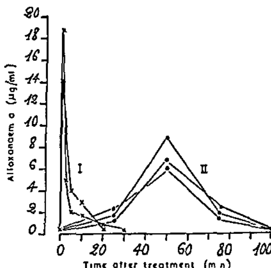


Fig 8

Comparative rates of development of hyperalloxanaem a in dogs w th exogenous and endogenous sources of alloxan

Non-anaesthetized dogs previously fasted 48 hours I injected with alloxan (50 mg per kg body wt i.v) II treated with 50 per cent glucose solution (2 mg per kg by mouth)

7 *Effect of pancreatectomy*

Hyperalloxanaemia was provoked by glucose in totally pancreatectomized dogs which had been fasted for three days The pancreas does not therefore seem to play a great part in the production of alloxan It was also produced in hypophyseal and metahypophyseal diabetic dogs

8 *Inhibition of alloxan synthesis*

We investigated the action of various substances on the hyperalloxanaemia induced in rats previously fasted for 48 hours (8, 9)

Table I summarizes the results obtained. It is interesting to note that insulin and phlorrhizin inhibited the phenomenon of provoked hyperalloxanaemia. The same was true of synthalin B, pentamidine, lomidine and *para*-aminobenzenesulphonamido-*iso*propylthiodiazole

TABLE I

Inhibition of alloxan synthesis

All rats fasted for 48 hours, then given a 50 per cent solution of glucose by mouth (2 g /kg body wt)

Inhibitor	*Rat no*	*Glycaemia (mg /100 ml)*		*Alloxan-aemia (µg /ml)*	
None	(Mean of 14 rats)	142		8.75	
Phlorrhizin (200 mg /kg by mouth at same time as glucose)	20	138	103	2.30	2.26
	21	—		2.32	
	22	132		1.70	
	31	81		1.10	
	32	60		0.90	
Phlorrhizin (200 mg /kg , subcut 30 min before glucose)	33	83	74	0.40	0.57
	34	65		0.51	
	35	—		0.80	
	36	—		0.50	
Insulin (1 unit/kg , subcut 90 min before glucose)	109	109	104	0.50	0.52
	110	106		0.50	
	111	95		0.56	
	112	105		0.50	
Synthalin B* (200 mg /kg , by mouth 3 hr before glucose)	86	131	108	1.60	1.61
	87	84		1.86	
	189	—		1.48	
	190	—		1.52	
Pentamidine† (10 mg /kg subcut , 3 hr before glucose)	84	139	131	0.60	0.66
	85	124		0.66	
Lomidine‡ (100 mg /kg subcut. 2 hr before glucose)	191	218	189	0.72	0.61
	192	160		0.53	
para-Aminobenzenesulphonamido*iso*propylthiodiazole (500 mg /kg subcut 3 hr before glucose)	81	308	277	0.80	0.51
	82	306		0.80	
	83	282		0.46	
	97	261		0.31	
	170	229		0.44	
	171	279		0.27	

* Decamethylenediguanidine dihydrochloride

† 4,4′-diamidino-α,ω-diphenoxypentane di(β-hydroxyethanesulphonate)

‡ 4,4′-diamidino-α,ω-diphenoxypentane dimethanesulphonate

9 *Comparison with the hyperalloxanaemia produced by injection of alloxan*

The degree and duration of the phenomenon of glucose-

aorta was 15–20 µg/ml. in the dog and 10–30 µg/ml in the rabbit The subsequent fall in concentration was rapid in both animals, ten to fifteen minutes after the injection, the alloxan level was very low and return to normal occurred rapidly Fig 8 shows the comparative rates of development of the hyperalloxanaemia when the source of alloxan was exogenous or endogenous

10 *Effect of hypophysectomy and adrenalectomy*

We recently investigated the effects of hypophysectomy and adrenalectomy on the hyperalloxanaemia which develops forty-five minutes after the ingestion of glucose by rats fasted for 48 hours (12) It was observed that adrenalectomy or hypophysectomy largely inhibited the effect (see Fig 10, part I)

On the basis of these experiments it is suggested that under ... prolonged fasting being only one

'hyperalloxanaemic shocks' of this kind may eventually injure the beta ... it may provisionally ... physiologically predispo... amounts of alloxan in the intestine

Another hypothesis may also be put forward that alloxan synthesized in the intestine may stimulate the secretion of insulin Although this hypothesis is an attractive one, we do not consider that there is as yet sufficient evidence in its favour However this may be, it is interesting to note that insulin will inhibit the formation, or favour the destruction of, alloxan originating in the intestine The action of phlorrhizin is more complex, but it is probable that it acts as a brake on the absorption of glucose and perhaps also facilitates the destruction of alloxan or its elimination by the kidneys

istics. It is possible that the hypophysis acts through the intermediary of the adrenal glands

HYPERALLOXANAEMIA AND DIABETES MELLITUS

Two theories have been put forward to explain how histological and functional alteration of the beta-cells of the islets of Langerhans occur during idiohypophyseal diabetes and become more pronounced during the evolution of metahypophyseal

attempt to control a sustained and semi-permanent hyperglycaemia. The *toxicity theory* of Houssay (3), on the other hand, attributes a particular toxicity for the beta-cells to the diabetogenic extract of the anterior lobe of the pituitary This latter mechanism may also play a part in the production of the metathyroid diabetes described by the same author

The toxicity theory has received valid support from the discovery of experimental diabetes following administration of alloxan. But the attempt to reconcile the two theories of how the islet lesions are established or aggravated in meta-alloxanic and metahypophyseal diabetes appeared somewhat daring and so far as we are aware had not been made prior to our own investigations (8, 9, 10, 11) The experiments which we now report, however, appear to plead in favour of such a single comprehensive hypothesis

extract (in dogs), metahypophyseal diabetes (in dogs), meta-

diabetes. Our observations were as follows·

1 Hyperalloxanaemia occurred in all types of diabetes mellitus except that provoked by injection of phlorrhizin. The

degree of alloxanaemia was in direct proportion to the severity of the diabetes, particularly in relation to the blood sugar level The level

ml of blc

feeding

for regulating their blood sugar level in these cases prolonged fasting lowered the level of alloxanaemia. *Thus, in serious cases of diabetes there is a permanent hyperalloxanaemia*

Parts II and III of Fig 10 show respectively the levels of alloxanaemia estimated in rats with meta alloxanic diabetes and

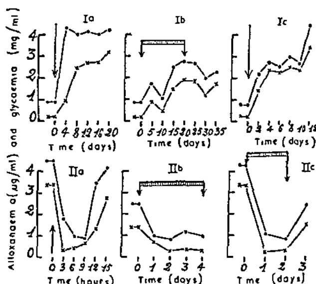


FIG 9

Alloxanaem a and glycaem a in dogs with pancreatic hypophyseal or meta-alloxan c d abetes

× — × alloxanaemia (μg /ml.) ● C

Ia 9 25 kg pancreatic d abetes

Ib 6 80 kg hypophyseal diabete-
betogenic anterior p tuitary extra
second arrow

Ic, 11 60 kg meta-alloxanic diabetes. (Arrow indicates i.v injectio i of 75 ml. per kg alloxan)

IIa 8 50 kg total pancreatic diabetes. (Arrow indicates subcut. injection of 12 units insulin.)

IIb 6 90 kg, metahypophyseal diabetes (Arrows indicate effect of 4 days fast 15 days after stoppage of treatment with anterior p tuitary extract)

IIc 7 70 kg total pancreatic diabetes (Between the two arrows 100 mg phlorrhizin injected subcut morning and evening)

in normal rats. Fig 9 (curves Ia, Ib, Ic) shows the increase in alloxanaemia in dogs following pancreatectomy, injection of an extract of the anterior lobe of the pituitary, and injection of a diabetogenic dose of alloxan

2. When a 'spontaneous cure' of diabetes mellitus occurred (meta-alloxanic diabetes in rabbits), the alloxanaemia returned to its normal level.

3. Insulin, either in a single dose or during prolonged treatment, reduced alloxanaemia to the normal level and maintained it there. Suspension of the injections had the opposite effect (Fig. 9, curve IIa)

FIG 10

Alloxanaemia and glycaemia in rats

I, following administration of 50 per cent solution of glucose (2 g /kg body wt, by mouth) to normal (*N*), hypophysectomized (*H*) and adrenalectomized (*S*) rats fasted for 48 hours

II [illegible] and

4. Prolonged fasting likewise reduced the hyperalloxanaemia in recently induced metahypophyseal or meta-alloxanic diabetes and at the same time the blood sugar returned to its normal level. It is probable that the effect was due to the persistence of the secretion of a certain amount of insulin (Fig 9, curve IIb)

5 Administration of phlorrhizin (5–10 mg/kg, morning and evening, subcutaneously) diminished the glycaemia in pancreatic diabetes and metahypophyseal diabetes in dogs and also lowered the level of alloxanaemia By this means the alloxanaemia could be maintained at the normal level for several weeks (Fig 9, curve IIc)

(Fig. 10, part II)

These, then, are the arguments provided by the experiments described in this section, which may answer the question 'Does alloxan play a part in the pathogenesis of diabetes mellitus?'

Our results certainly appear to point to an affirmative answer If it can be confirmed that it is alloxan itself which is being estimated, one could find satisfactory answers to the following questions

1 Why long-sustained hyperglycaemia may facilitate the occurrence of diabetes mellitus,

2 Why (at least in certain species) permanently established diabetes becomes progressively more severe in the majority of cases,

3 Why treatment with insulin or phlorrhizin protects the islets against the occurrence of lesions (in the initial stage of permanent diabetes) or why an aggravation of these lesions recurs in 'meta' diabetes (metahypophyseal, meta-alloxanic or metathyroid),

favour the

diabetes (metahypophyseal or meta-alloxanic),

groups to the

shment

ive per

cent of the pancreas

of the

[illegible]

for anti-alloxanic pharmacodynamic substances (inhibiting its production, favouring its destruction, or opposing its ill-effects), with which to break this vicious circle.

Research is already in progress, but it is not yet possible to foresee the consequences of its development

REFERENCES

1. ARCHIBALD, R M, *J biol Chem*, 158, 347 (1945)
2. HOUSSAY, B A, *Endocrinology*, 30, 884 (1942)
3. HOUSSAY, B A, *J Physiol Path gen*, 39, 249 (1942)
4. HUISMAN, T. H J, VAN HULTEN, A and STEL, H, *Biochim biophys Acta*, 6, 289 (1950)
5. VON LIEBIG, J, *Liebigs Ann*, 121, 80 (1862)
6. LOUBATIÈRES, A, *Montpellier méd*, 19-20, 345 (1941)
7. LOUBATIÈRES, A, *Ann Nutr*, *Paris*, 1, 571 (1947)
8. LOUBATIÈRES, A and BOUYARD, P, *C R Acad Sci*, *Paris*, 232, 257, 1251 (1951)
9. LOUBATIÈRES, A and BOUYARD, P, *C R Soc Biol*, *Paris*, 145, 344, 493 (1951)
10. LOUBATIÈRES, A. and BOUYARD, P, *J Physiol*, *Paris*, 43, 786 (1951)
11. LOUBATIÈRES, A and BOUYARD, P, *C R Soc méd Montpellier*, meeting of 7 July 1950, publ in *Montpellier méd*, 49-50, 56 (1951)
12. LOUBATIÈRES, A, STACK-DUNNE, M P and BOUYARD, P, unpublished experiments
13. LUKENS, F D W and DOHAN, F C, *Endocrinology*, 30, 175 (1942)
14. LUKENS, F D W, DOHAN, F C and WOLCOTT, M W, *Endocrinology*, 32, 475 (1943)
15. PETIT, L, *Ann Agron*, *Paris*, 1, 1 (1950)
16. SCHOLER, P, *Biochim biophys Acta*, 2, 260 (1948)

DISCUSSION

YOUNG Am I correct in [illegible] [illegible]

LOUBATIÈRES It will be difficult for a long time to come to extract alloxan in its pure state from a biological medium because it decom-

poses so rapidly For this reason we have attempted to arrest its breakdown at a point as near as possible to the start In spite of the difficulty of this, it has been possible to show by fluorescence spectroscopy that the supernatant remaining after deproteination of the blood contains a substance whose structure is closely related to that of alloxan

LAZAROW Do dehydroascorbic acid or dialuric acid react with *ortho*-*phenylenediamine under these conditions?* Since dialuric acid, the reduction product of alloxan, is much more stable at pH 7 4 than is alloxan, I wonder whether you might be estimating dialuric acid. If you are estimating alloxan, then the amount of alloxan formed would be very much greater than the amount indicated by your assay Since the half-life of alloxan at 37°C and pH 7 4 is about one minute much of the alloxan formed would be destroyed unless, of course, the alloxan is protected in some way or reduced to dialuric acid

LOUBATIÈRES I am equally surprised that it is possible to detect alloxan in the blood in amounts as great as 4 µg per ml, for its disappearance must be very rapid It must be borne in mind, however, that these amounts are found in a permanent state only in the blood of diabetic animals The determinations have been made by a physical method which, though possibly less specific, is certainly more sensitive than the chemical methods at present available

Uric acid does not gives any fluorescence with *ortho*-phenylenediamine, according to Archibald I do not know what happens with dialuric acid

GAARENSTROOM An attempt has been made by Huisman van Hulten and Stel, in my laboratory, to detect alloxan by Archibald's method after treatment of the animals with glucose, but with no success Perhaps this is due to the extraordinary rapidity of determination by Professor Loubatieres, he centrifuged for only 30 seconds

LOUBATIÈRES I think that the failure of Professor Gaarenstroom and his collaborators to confirm our results was for the following reasons (i) it is necessary to work very quickly, to withdraw the blood very rapidly, and to trap the alloxan, (ii) the method known as internal standardization must be used and *I do not think that they did this* (iii) variations in the intensity of the light source, caused by fluctuations in the mains supply, must be eliminated, (iv) the correct filters must be used Dr Gaarenstroom and his collaborators probably did not use the filters mentioned by Archibald As far as I can remember, they were filters used for the estimation of vitamin A Their origin and number should have been defined We know from our own experience that the use of incorrect filters may lead to serious errors

LONG What is your procedure for concentrating the blood 200-300 *times, Professor Loubatières?*

LOUBATIÈRES This has not yet been published As the alloxan derivatives can be trapped with the reagent used for the estimation, we add the blood directly to this reagent Concentration of the solution is effected by evaporation at 60°C The mixture is reduced to small volume and treated with alcohol It is then taken to dryness and on redissolving in distilled water gives a solution which may be concentrated as much as required The fluorescence spectrum can be observed at each stage of the manipulations

LONG Do you know whether any alloxan is lost during this treatment?

LOUBATIÈRES Yes, there is a considerable loss of material during these operations

LAWRENCE How much blood do you need for this assay?

LOUBATIÈRES 3–4 ml of blood

LUKENS Has the normal occurrence of alloxan in the blood been demonstrated by any other method?

LOUBATIÈRES According to Archibald s paper and also to our own tests other methods are not sufficiently sensitive Even the largest amounts found in our experiments are too small to be shown up by other methods

WILHELMI I should like to inquire about your control experiments All your experiments showed alloxanaemia after glucose treatment Have you used protein or an amino-acid hydrolysate, or even fat, to see if the effect is still obtained?

LOUBATIÈRES We have started work on this problem and experiments are at present in progress It would certainly be interesting to know whether the simultaneous administration of glucose and fat or protein would inhibit or accentuate the effects

LUKENS In connection with Professor Loubatieres' report on the presence of alloxan in blood, our studies on the possible occurrence of a derivative of alloxan in urine may be mentioned We have already been reminded of the conversion of alloxan to alloxanic acid or its salts, alloxanates, in the presence of alkali Seligson found that hot alkaline hydrolysis (at 15 lb pressure) broke alloxanic acid down to mesoxalic (ketomalonic) acid The colorimetric determination of mesoxalic acid which he described provides an indirect measurement of alloxanic acid When this method is applied to urine the results are little better than qualitative nevertheless, two principal facts have emerged —

(1) As the following table shows normal urine contains a substance or substances which yield mesoxalic acid on alkaline hydrolysis As the conditions of the experiments served to eliminate uric acid as the *precursor, it is possible that alloxanic acid or some related pyrimidine*

may be the source of the mesoxalic acid found The wide range of values found from day to day must be used to emphasize the essentially qualitative nature of the results Examination of the data for different species suggests that there is a relation between body weight and the amount of this product that is formed

Amounts of mesoxalic acid obtained from urine after hydrolysis

Source	No of subjects	No of determinations	Mesoxalic acid per day	
			Mean (μmoles)	Range (μmoles)
Normal humans	5	10	217	92–331
Diabetic patients	5	9	414	109–1062
Normal cats	5	16	8 4	1 7–20 7
Normal rats	16	16	1 7	0 5–4 9

(ii) When alloxan (or sodium alloxanate) was administered to rats or cats, the urinary mesoxalic acid concentration was increased twenty-fold or so in the first twenty-four hours This increase was not accompanied by any change in uric acid excretion and it did not occur after the administration of diabetogenic doses of dehydroascorbic acid

The excretion of greater quantities of a precursor or precursors of mesoxalic acid after the administration of alloxan and the occurrence

alloxanaemia is suppressed A similar inhibition occurs if certain sulphonamides are administered at the same time as the glucose It is possible that part of the alloxan formed originates from certain intestinal micro-organisms However, no alloxan is elaborated by cells of the intestinal mucosa which have been carefully washed and then placed in Tyrode solution Yet as soon as glucose is added alloxan is produced

DE JONGH Do you think that alloxanaemia should be considered as a symptom or as a cause of the disease of diabetes?

LOUBATIÈRES A certain amount of reserve *is necessary* inasmuch as we are not sure that the substance in question is *really alloxan* It is tempting to suppose that, for a certain time prior to the onset of

diabetes, hyperalloxanaemia exists which causes first a stimulation and subsequently a lesion of the islets. It is interesting to note that in hypophysectomized fasting rats, the wave of hyperalloxanaemia which occurs following the administration of glucose is reduced in height T[illegible]
diabetic a[illegible]
ectomy[illegible]
theless apparent, is established

LAZAROW If I may refer again to the point raised by Professor [illegible]

[illegible] carbohydrate metabolism Is there any indication that pyrimidine metabolism is linked with carbohydrate metabolism?

LOUBATIÈRES I am unable to answer that question at present.

effects upon carbohydrate metabolism in normal humans (8 9, 11) We reported the following observations

1 That with large daily doses of ACTH a state of diabetes characterized by hyperglycaemia and glycosuria can be induced in normal people

2 The type of diabetes induced is relatively resistant to the hypoglycaemic effect of exogenous insulin

3 Simultaneous administration of insulin along with the ACTH does not interfere with the development of the diabetic state

4 *Although hyperglycaemia is present, diminished renal* tubular absorption of glucose is in part responsible for the glycosuria

5 Some normal individuals exhibit glycosuria without hyperglycaemia in response to ACTH

6 Although

tive nitrogen

by ACTH ... is not accountable *per se for pro ... of the diabetic state*

7 It was suggested that interference with peripheral utilization of glucose (decreased oxidation and/or diminished conversion to fat) is in part responsible *for the production* of steroid diabetes

8 *That a fall in the concentration of blood glutathione is* associated with the induction of steroid diabetes in man Because of the interesting triad of events occurring simultaneously under *the influence of ACTH, namely, diabetes mellitus, increased* uric acid excretion and decreased concentrations of blood glutathione, it was speculated that in addition to the mechanisms indicated above, the beta-cells of the islets of Langerhans might be exposed to an alloxan-like intermediary, with a resultant functional impairment *in insulin production*

9 When assayed on the basis of adrenal ascorbic acid depletion, some batches of ACTH appear to be much more diabetogenic *in man* than others This suggested that there might exist more than one type of ACTH or that the ascorbic acid depletion assay did not run parallel with *all of the metabolic effects as they* are observed in man

That an ... humans is ... CTH and

by 11 17-oxycorticosteroids is evidenced by a number of other clinical observations Administration of either ACTH or cortisone to patients with pre-existing diabetes intensifies the diabetic state and sometimes increases the insulin requirement significantly (2, 4, 17, 25) It has also been observed that cortisone lessens the sensitivity to insulin of patients suffering from Addison's [illegible]

suggested that a diminished functional reserve of islet tissue accounts for the more intense diabetogenic activity of ACTH or cortisone in one apparently normal individual as opposed to another (28) Thus, Sprague noted that two of four patients who exhibited impairment of carbohydrate tolerance under the influence of cortisone had diabetic family histories (29) While the reserve capacity of the pancreas for insulinogenesis must be important in this regard, we are somewhat perplexed by some recent observations made in our own laboratory (12) When a single injection of 100 mg of ACTH is given to mild diabetics who do not require insulin, the glucose tolerance test does not change significantly from the control curve On the other hand, when the same procedure is carried out in normal individ [illegible] majorit [illegible] ance I [illegible] by the administered ACTH

DIABETOGENIC EFFECT OF ADRENAL CORTICAL STEROIDS

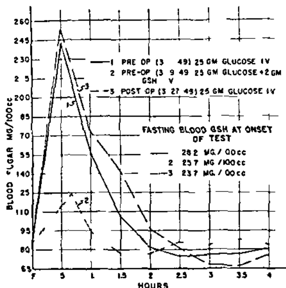
In man it has been difficult to evaluate precisely the relative diabetogenicity of the pure adrenal cortical steroids Our own experience and that of others would indicate the following order of decreasing diabetogenic potency in apparently normal humans 17-hydroxycorticosterone (hydrocortisone), cortisone, corticosterone, 11-dehydrocorticosterone and 11-desoxycorticosterone The last named has no demonstrable effect upon carbohydrate metabolism and 11-dehydrocorticosterone exerts a very mild, if significant, effect (5, 6, 8, 13, 26)

ADRENAL STEROID DIABETES AND THE BLOOD GLUTATHIONE LEVEL

Because the true significance of the fall in the concentration of blood glutathione induced by ACTH hydrocortisone and cortisone remains obscure the phenomenon should receive further consideration Our group has observed that during the induction of diabetes in normal people with either ACTH or hydrocortisone the concentration of glutathione in the red blood cells diminishes (8, 9 11) This observation has been confirmed in man by Hess *et al* (16) but Sprague observed no such changes (29) More recently it has been found that diabetes induced with cortisone in rats is associated with a significant fall of the blood glutathione level and that the sulphydryl content of the tissues themselves is diminished by excessive adrenosteroidal activity (15 23) Further, it has been reported that intravenous administration of reduced glutathione in the course of induction of steroid diabetes in man results in a transient alleviation of the diabetic state (10) On the other hand, Lazarow has observed intensification of cortisone-induced diabetes in rats when reduced glutathione was administered (23) At present the divergent findings in the two species cannot be reconciled

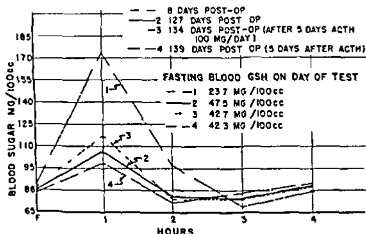
Whether or not the fall in the blood glutathione concentration reflects increased activity or production of an alloxan-like intermediary in the tissues remains to be established by future work. That the decreased concentration of blood glutathione does however, appear to be related to the presence of diabetes is suggested by our further observations Figs 1 and 2 represent data obtained from a case of Cushing's syndrome with diabetes in which the diabetes disappeared following subtotal adrenalectomy It will be observed in Fig 1 that the blood glutathione level was subnormal at the onset of each of the three glucose tolerance tests Diabetes existed before operation and for a short period of time after operation However, in the pre-operative period when two grams of reduced glutathione were given intravenously along with the glucose a normal curve was obtained It will be seen from Fig 2 that by 127 days after operation the fasting blood glutathione had returned to a normal value and the subsequent glucose tolerance tests were normal

In contrast we wish to show the findings on another case of Cushing s syndrome in which diabetes was not present That



FIG

I ffec f ntravenous r ed ced gl a t e (GSH) upon a t hyd a e t lera e n C sl ng s sy f n



Di ppea ance of d abetes and ncrease of b d g a t e (GSH) oncentra on fol wing s b tal l era al ct my C h ng s syndr n

Test 25 g glucose i v

this case presented the classical picture of Cushing's syndrome

tration was consistently at the upper limits of normal It is also



Fig 3

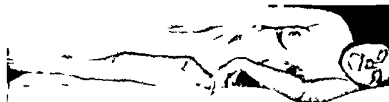


Fig 4

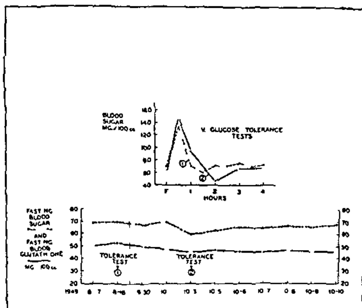


FIG 5
Cush ng's syndrome with ı ormal carbohydrate tolerance and h gh blood glutath one concentrat on

to be observed that the results of both glucose tolerance tests fall within the normal range

SUMMARY

1 When administered in large doses to man, ACTH is capable of inducing a diabetic state characterized by hyperglycaemia, glycosuria, decreased renal tubular reabsorption of glucose and relative insulin resistance It is the same type of diabetes which occurs spontaneously in Cushing's syndrome

2 Adrenal cortical steroids of the 11-oxygenated type produce a similar type of disturbance in carbohydrate metabolism when administered to man In descending order of diabetogenic

potency they are 17-hydroxycorticosterone (hydrocortisone), cortisone, corticosterone and 11-dehydrocorticosterone

3 Steroid diabetes in man is associated, initially at least, with marked catabolism of body protein However, glyconeogenesis from protein cannot account alone for the diabetes which is observed Interference with the normal pathways for disposal of glucose appears to play a major role

4 While resistance to exogenous insulin can be demonstrated in steroid diabetes, it is not known whether the pancreas under conditions of steroid diabetes is secreting subnormal, normal or supranormal quantities of insulin

[illegible]

excess of carbohydrate-active adrenal steroids

6 ACTH, hydrocortisone and cortisone are capable of reducing the level of blood glutathione in man This phenomenon appears to be related to the appearance of the diabetic state A similar relationship exists in Cushing's syndrome with diabetes The precise significance of this relationship must await further investigations

REFERENCES

1 BENNETT, L L and LI, C H, *Amer J Physiol*, 150, 400 (1947)
2 BROWN E M Jr, LUKENS, F D W, ELKINGTON, J R and DEMOOR, P, *Endocrinology*, 10, 1363 (1950)
3 BROWNE, J S L., in *Conference on Metabolic Aspects of Convalescence*, ed REIFENSTEIN E C, Jr, New York Josiah Macy, Jr Foundation (1943)
4 BOLAND, E W and HEADLEY, N E, *J Amer med Soc*, 141, 301 (1949)
5 CONN, J W, in *Adrenal Cortex, Transactions of the First Conference*, ed RALLI, E P, New York Josiah Macy, Jr Foundation (1950)
6 CONN, J W, in *Adrenal Cortex, Transactions of the Second Conference*, ed RALLI, E P, New York Josiah Macy, Jr Foundation (1951)
7 CONN, J W, FAJANS, S S and LOUIS, L H, *Trans Ass Amer Phycns*, 64, 269 (1951)

8 CONN, J W, LOUIS, L H and JOHNSTON, M W, *Proc Amer Diabetes Ass*, 8, 215 (1948)
9 CONN, J W, LOUIS, L H and JOHNSTON, M W, *J Lab clin Med*, 34 255 (1949)
10 CONN J W, LOUIS, L H and JOHNSTON, M W, *Science*, 109 270 (1949)
11 CONN, J W, LOUIS, L H and WHEELER, C E, *J Lab clin Med*, 33, 651 (1948)
12 FAJANS S S and CONN, J W, unpublished experiments
13 FORSHAM, P H, THORN, G W, BERGNER, G E and EMERSON, K, Jr, *Amer J Med*, 1, 105 (1946)
14 FORSHAM, P H, THORN, G W, FRAWLEY, T F and WILSON, D L, *J clin Endocrin*, 10, 825 (1950)
15 GOLDZIEHER, J W, RAWLS, W B and GOLDZIEHER, M A, presented at the Annual Meeting of the Endocrine Society, Chicago, June 1952
16 HESS, W C, KYLE L H and DOOLAN, P D, *Proc Soc exp Biol, N Y*, 76 418 (1951)
17 INGLE, D J, *Proc Soc exp Biol, N Y*, 44, 176 (1940)
18 INGLE, D J, *Publ Amer Ass Advanc Sci (The Chemistry and Physiology of Hormones)*, p 83 (1944)
19 INGLE, D J, LI C H and EVANS, H M, *Endocrinology*, 39, 32 (1946)
20 INGLE, D J, SHEPPARD, R. EVANS, J S and KUIZENGA, M H, [illegible]
2[illegible] KUIZENGA, M H, [illegible]
2[illegible] *Physiol*, 132, 670 (1941)
23 LAZAROW, A, *Diabetes*, 1, 171 (1952)
24 LONG, C N H, KATZIN, B and FRY, E G, *Endocrinology*, 26, 309 (1940)
25 PERERA, G A, PINES, K L, HAMILTON, H B and NISLOCKY, K, *Amer J Med*, 7 56 (1949)
26 SPRAGUE, R G, personal communication
27 SPRAGUE, R G, KEPLER, E J, KEATING, F R and POWER, M H, *J clin Invest*, 26, 1198 (1947)
28 SPRAGUE R G, MASON H L and POWER, M H, *Proc Amer Diabetes Ass*, 9, 149 (1949)
29 SPRAGUE, R. G, POWER, M H., MASON, H L, ALBERT, A, MATHESON, D R, HENCH P S, KENDALL, E C, SLOCUMB, C H and POLLEY, H F, *Arch intern Med*, 85 199 (1950)

DISCUSSION

Lawrence Are they true glucose values in the last case which you described (Fig 5), Dr Conn?

Conn Yes

Young Did you find any effect of the composition of the diet on the response to ACTH?

Conn This was not studied It has been reported that a diet low in sodium may influence the carbohydrate effect of ACTH

Lukens Your first patients treated with ACTH, Dr Conn, received a diet containing 3000 kg cal But in experiments by Thorn a diet containing only 1500 kg cal, was used and no glycosuria was produced The total caloric intake may therefore be very important Many of your patients developed glycosuria, but many hundreds of patients with rheumatoid arthritis have been treated with cortisone or ACTH without any response being elicited I am wondering, therefore, whether some of your patients were selected

Conn At first we thought we had discovered a possible method of selecting men who would react unusually strongly to ACTH, but subsequent analysis showed that this idea was incorrect That is, [illegible]

Best Concerning the doses of insulin which Dr Conn found were [illegible]

ACTH in man?

Conn Inasmuch as the effect of ACTH on carbohydrate metabolism is peripheral, I suspect that the diabetogenic action could not be prevented entirely with insulin

Best The evidence is not very convincing *Much* larger doses of insulin might prevent it Would it be safe to give such large doses?

Long Ingle gave 1000 units of protamine zinc insulin to rats treated with ACTH

Have there been any studies of the effect of ACTH or cortisone on the insulin content of the pancreas?

Young Evans observed an increase in the insulin content of the pancreas when ACTH was administered

Wilhelmi But under the conditions of Evans' experiments no diabetogenic effects were produced

Best Haist in our laboratory *has found that treatment of rats with*

cortisone increases the volume of the islets Perhaps the insulin content increases in the same proportion

LUKENS. Is there any insulin-like effect of cortisone in patients with pituitary insufficiency? Are there any indications of synergism?

CONN No

HOET I saw a case of panpituitary insufficiency which was very sensitive to cortisone The cortisone was also very effective in abolishing the insulin sensitivity.

LONG Has Dr Conn made any study of the alteration in renal reabsorption of glucose? Lambert and his colleagues found that this was unchanged after administration of ACTH

CONN. There is much evidence on both sides of the question, but I have not studied it Clinically, a mild glycosuria can be present without hyperglycaemia in patients treated with ACTH or cortisone for rheumatoid arthritis

HOET The activity of administered cortisone is very much greater in cases of Addison's disease than in balanced patients Patients who [illegible]

[illegible]

active adrenals

CONN It is true that a patient with Addison's disease does show a much greater response to cortisone than does a normal individual In fact, a dose of 100 mg per day is not tolerated very long in a patient with Addison's disease Even 50 mg per day for a week would not be tolerated much longer.

LAWRENCE Do cases with Cushing's syndrome ever get ketosis?

CONN Very seldom—and then it is very mild We have never seen [illegible]

[illegible]

possible that alloxan-like compounds are derived from uric acid, I believe that pyrimidines are a much more important source of alloxan than uric acid The lysis of tissue which follows the administration of ACTH results in a greater breakdown of protein and nucleic acid The purines thus liberated are excreted as uric acid, but the pyrimidine components of the nucleic acid could give rise to alloxan This prob- [illegible]

[illegible]

needed at least 60 units of insulin There was no constant increase in

the insulin requirement when ACTH or cortisone was given, but it seemed to vary a little from case to case

CONN My experience is similar In some cases the dose of insulin must be increased fourfold and in others not at all

LAWRENCE Have you had any cases of permanent diabetes caused by administration of these compounds? I have come across one (BISHOP, P M F and GLYN, J H, *Proc R Soc Med*, 45, 169, 1952) This was a man aged 31 Glycosuria was absent, although no glucose tolerance curve was obtained before the administration of cortisone After treatment for four days he had a heavy glycosuria The cortisone administration was stopped after a week and the blood sugar level was then 300–400 mg per cent, this persisted He needed 100 units of insulin There was no accompanying ketosis We hope to do further experiments with him

CONN Several cases of diabetes have been reported following cortisone administration Many of these are probably potential diabetics

HOET A single dose of 100 mg of cortisone would give quite a good test of the state of the pancreas of the patient A hyperglycaemic response would indicate that the pancreas has a reduced capacity for producing insulin

HANSSEN Have you any comments to make on the use of ACTH as a diagnostic test for incipient diabetes Dr Conn?

CONN For the past two years we have been studying the children of the diabetics coming to our clinic We first of all carried out a glucose tolerance test We then gave one dose of 100 mg of ACTH Then at 8 a m the following day we performed a second glucose tolerance test. So far it has been difficult to evaluate the results We found as many potential diabetics in the controls (children of non-diabetics) as in the others We will have to carry on the experiment for a period of about twenty years or more before being able to say whether or not such a test will identify potential diabetics

BEST The results may become more interesting later

CAMPBELL Was there any indication that different preparations of ACTH elicit different types of response—effects on the eosinophil count or the blood sugar level, for example?

CONN We used the intact ACTH molecule With two different batches of ACTH which had been assayed by Armour & Co the negative nitrogen balance was the same with the two preparations the eosinopoenia was the same, but the diabetogenic effect was different Both batches were tested in a number of individuals

CAMPBELL Can the preparations be grouped according to the adrenal ascorbic acid or diabetogenic response?

CONN The more consistent response was the negative nitrogen balance

YOUNG Some of the commercial preparations are definitely partially hydrolysed by acid.

CONN Some of the later preparations I used certainly were

YOUNG Can you suggest a figure for the relative activities on carbohydrate metabolism of 17-hydroxycorticosterone and cortisone?

CONN It is very hard to make a numerical evaluation on the basis of experiments in man but very rough figures for the effects of cortisone and 17-hydroxycorticosterone would be as 1 1 4–1 5

LONG Many years ago Fry and I obtained the ratio of 1 2

RUSSELL We had similar figures

THE ADRENAL CORTEX AND CARBOHYDRATE METABOLISM

By

F VERZÁR

Physiologisches Institut der Universitat, Basel Switzerland

INTRODUCTION

A decrease in the glycogen content of liver and muscle and hypoglycaemia were already recognized as symptoms of the later stages of adrenal cortical insufficiency in the early period of the study of adrenal cortical physiology by Britton and his colleagues, in the years 1932–1938 (see ref 4) When, however, the disturbances of the electrolyte metabolism became known, the primary importance of the changes in carbohydrate metabolism was questioned The availability of adrenal cortical extracts and of corticosteroids was responsible for the commencement of comparative studies on the metabolism of carbohydrate and of sodium potassium and water

STIMULATION OF GLYCOGEN PRODUCTION

sterone acetate (DOCA) (70) [illegible]
tained normal amounts of glycogen their blood sugar was normal and adrenaline gave a normal hyperglycaemic curve There was still a hypersensitivity to insulin But the physio- [illegible] and lactation was completely normal [illegible] ve with [illegible] carbo-

Several observations showed us, and others, that when compared with the lipid extracts of Swingle and Pfiffner, and later with other corticosteroids, there were certain differences in action.

Adrenal corticosteroids were originally tested by their effect on muscle work The marked adynamia of the adrenalectomized animal was demonstrated in the early work of Csik (1930)

shown to run parallel to that on glycogen production by the liver in the 'six-hour test' of Reinecke and Kendall and Olson *et al* (54, 59) It seemed plausible that the disturbances of glycogen metabolism were responsible for the decrease of muscular work The life- and growth-preserving activity is, however, highest with DOCA, which had small or even no activity in the two previously mentioned tests

On this basis, a differentiation was made between the corticosteroids which increased the amount of liver glycogen in the fasted rat in a six-hour test, and those which did not The former were called 'gluco-corticosteroids', the latter, 'mineralo-corticosteroids' The former are 11-oxycorticosteroids, the latter are represented by desoxycorticosterone acetate, which, even in the smallest quantities maintains the metabolism of sodium, potassium and water, and also the life of the animals

As we had found in 1940–1941 that the glycogen content of liver and muscle was normal in cats and in rats kept alive with DOCA, we concluded that no qualitative differentiation should be made between these two groups (76) We showed that the so-called mineralo-corticosteroid DOCA has a very distinct glycogenetic activity if it is given continuously (60, 61) Obviously it did not act as quickly as adrenal cortical extract or cortisone, particularly in the six-hour test

For example, adrenalectomized rats were kept on a pure protein diet, then fasted for twenty-four hours before being injected with the substances indicated in Table I Liver glycogen was assayed at the times indicated It is obvious from the Table that DOCA was active, though much less than cortisone

If adrenalectomized rats were kept on a mixed stock diet and treated daily with DOCA from the day of adrenalectomy, the values of liver glycogen given in Table II were observed in the non-fasting animals (1½ hours after the food was taken away)

In a similar experiment the animals were kept for several weeks on a pure protein diet The liver glycogen values were

TABLE I

Effect of corticosteroids on liver glycogen content

(The numbers of animals used in each case are given in brackets.) (from ref 60)

Time after injection (hours)	Liver glycogen content (g /100 g) Desoxycorticosterone acetate (2 mg)	Cortisone (2 mg)	Adrenal cortical extract (Upjohn) (3 ml.)
6	0 12 (3)	1 75 (2)	0 14 (2)
12	1.28 (2)	3 08 (3)	1 16 (2)
24	46 (3)	4 30 (2)	0 27 (2)

TABLE II

Effect of desoxycorticosterone acetate (DOCA) on liver glycogen content of animals on mixed diet

(from ref. 61)

Condition	Number of animals	Treatment	Liver glycogen content (g /100 g)
Normal	18	none	4.8 ± 0 3
Adrenalectomized	11	none	1 5 ± 0 4
Adrenalectomized	10	DOCA (2 mg for 2–5 days)	4.7 ± 0 6
Adrenalectomized	15	DOCA (2 mg for 10–20 days)	4.8 ± 0.4

TABLE III

Effect of desoxycortiscosterone acetate (DOCA) on liver glycogen content of animals on protein diet.

(from ref. 61)

Condition	Number of animals	Treatment	Liver glycogen content (g /100 g)
Normal	7	none	3 54 ± 0 96
Adrenalectomized	14	none	0 08 ± 0 016
Adrenalectomized	13	DOCA (3 mg)	2 28 ± 0 18

TABLE IV

Effect of desoxycorticosterone acetate (DOCA) and cortisone on liver glycogen content of animals on protein diet

(from ref 61)

Daily treatment	Days of treatment	Number of animals	Liver glycogen content (mean values) (g /100 g)
DOCA (2 mg)	13	23	0 89
DOCA (2 mg)	14	20	2.26
DOCA (3 mg)	13	13	2 28
DOCA (4 mg)	16	10	1.20
Cortisone (1 mg)	16	10	1 90
Cortisone (2 mg)	14	11	3 57

lower in the adrenalectomized animals, but again the DOCA-treated animals had almost normal values (Table III)

Thus, in animals continuously treated and fed, there is little difference between the activities of 11-oxy- and 11-desoxycorticosteroids on liver glycogen But cortisone certainly acts in smaller doses and can raise the glycogen values higher, as Table IV shows for similar series of adrenalectomized rats on a pure protein diet.

In view of the action of corticosteroids on carbohydrate metabolism, it seems important to us that their activity can be demonstrated not only in continuously treated, fasted and non-fasted animals, but also in animals treated in the following way adrenalectomized animals, previously kept on a mixed diet and then fasted for twenty-four hours, received corticosteroid and, one hour later, 1 g (*i e* 2 ml 50 per cent) glucose by stomach tube Table V shows that the liver glycogen content of DOCA-treated animals increased as much as in normal animals, whereas the untreated animals showed only a very small increase

TABLE V

Effect of desoxycorticosterone acetate (DOCA) on liver glycogen content when 1 g glucose given after 24 hours starvation

(from ref. 61)

Condition	Number of animals	Treatment	Liver glycogen content after 3 hours (g /100 g)
Normal	6	none	1 3 ± 0 18
Adrenalectomized	5	none	0 4 ± 0.22
Adrenalectomized	6	DOCA (2 mg)	1 7 ± 0 18

This experiment was repeated by Wang with DOCA and cortisone for comparison (86) Adrenalectomized rats and normal controls fasted for twenty-four hours, received the corticosteroid, one hour later they were fed 1 g glucose, and three hours later the glycogen was estimated The results (Table VI) show clearly that under these experimental conditions little difference exists between DOCA and cortisone when glycogen is produced from the glucose which is fed

These last experiments prove that corticosteroids also increase the amount of glycogen produced from glucose This supports the view of Engel *et al* that neither the protein molecule nor deamination of amino-acids are the point of action of the cortico-

steroids (15) We may state, therefore, that corticosteroids enhance glycogen formation in the adrenalectomized animal 11-*Desoxycorticosterone has a much slower* action than the 11-oxycorticosteroids, of which cortisone is especially active, in the protein-fed fasting animal

TABLE VI

Effect of desoxycorticosterone acetate (DOCA) and cortisone on liver and muscle glycogen levels when 1 g glucose given after 24 hours starvation.

(from ref. 86)

Condition	Number of animals	Liver glycogen content (g/100 g)	Muscle glycogen content (g/100 g)
Normal starved 24 hr	5	0 10 — 0 12	0 19 — 0 34
Normal 3 hr after 1 g glucose given	5	3 70 — 5 70	0 45 — 0 55
Adrenalect untreated, 3 hr after 1 g glucose given*	3	0 92 — 1 43	0 37 — 0 45
Adrenalect treated with cortisone†	3	4 75 — 7 00	0 56 — 0 59
Adrenalect treated with DOCA‡	3	3 80 — 5 50	0 45 — 0 73

*10 12 days after adrenalectomy untreated.
†8 days after adrenalectomy treated with 0 5 1 0 or 2 0 mg cortisone daily
‡15 days after adrenalectomy treated with 1 mg DOCA daily

Nissim has recently calculated the relative activities of desoxycorticosterone and cortisone (53) How far such a calculation [illegible] involved [illegible]

to 11 oxycorticosteroids *in the body by the adrenals, liver and other tissues* This *may* account for the delayed effect of these substances on carbohydrate metabolism (21, 30)

TABLE VII

Effect of different doses of desoxycorticosterone acetate (DOCA) on liver and muscle glycogen content in normal rats starved for 24 hours

(from ref. 80)

Treatment	Liver glycogen content (g/100 g)	Muscle glycogen content (g/100 g)
None	0 10	0 25
After 1 g glucose*	3 31	0 55
After 1 g glucose and 2 mg DOCA†	1 89	0 44
After 1 g glucose and 20 mg DOCA†	0 71	0 25

*Killed 3 hours after 2 ml. 50 per cent glucose was given by stomach tube
†1 hour before glucose was given, DOCA was injected subcutaneously

INHIBITION OF GLYCOGEN PRODUCTION

By administering different quantities of DOCA to normal and adrenalectomized rats fasted for twenty-four hours, we noticed [illegible]

[illegible]

(narcosis-like) as Selye described (64) (Table VII) Whether this breakdown of cell-reserves is responsible for the state of intoxication, may be questioned

This adverse action of corticosteroids—the inhibition of glycogen metabolism—was first found when working with the isolated diaphragm of the rat (81) This muscle, in Ringer's solution, produces large quantities of glycogen from glucose if 1–10 units of insulin per 100 ml are present Koepf *et al* noticed that the diaphragms of adrenalectomized rats also synthesized glycogen from glucose in the presence of insulin (32) It was concluded, therefore, that adrenalectomy does not seriously disturb the synthesis of glycogen from glucose However, we found that in rest or work, and with or without glucose (100–400 mg per 100 ml), the adrenalectomized animal's muscle always metabolized significantly less glycogen than that from a normal animal (48, 49) (Table VIII)

Minced liver or muscle from adrenalectomized animals produces glycogen from glucose-1-phosphate Thus the main point of action of the corticosteroids cannot lie between glucose-1-phosphate and glycogen (83) But the undamaged diaphragm is unable to use glucose-1-phosphate This might indicate that this molecule does not enter into the muscle by diffusion, however, other explanations are also possible

We were rather puzzled when we found that the addition of corticosteroids to the isolated diaphragm does not increase the rate of glycogen formation, but on the contrary inhibits the metabolism of glycogen under all those conditions which were studied (49, 82, 84) (Table IX) The inhibition was found primarily with desoxycorticosterone The inhibitory doses were 1–10 µg per 100 mg diaphragm, that is, 1–10 mg per 100 g of muscle (100 g is about the quantity of muscle in a 200–300 g rat, which after adrenalectomy needs 2–3 mg of desoxycorticosterone per day) The inhibition was present in a normal as well as in an

TABLE VIII

Glycogenesis, glycogenolysis and glycogen consumption of rat diaphragm in rest and work

(from ref 49)

Additions to Ringer's solution.	Normal					Adrenalectomized				
	Glycogen (mg/100 g)			Work (cm^3/100 g muscle)	No of expts	Glycogen (mg/100 g)			Work (cm^3/100g muscle)	No of expts
	Change		Consumed during work			Change		Consumed during work		
	Rest	Work				Rest	Work			
None	−85	−245	160	27	25	−40	−145	105	22	10
Glucose (100 mg/100 ml)	+35	−145	180	32	23	+45	−50	95	36	8
Glucose (400 mg/100 ml)	+80	−110	190	41	10	+60	−35	95	28	5
Glucose (100 mg/100 ml) + insulin (1 unit)	+170	−90	260	36	11	+90	−40	130	32	6
Glucose (400 mg/100 ml) + insulin (1 unit)	+365	+10	355	38	16	+290	+80	210	28	11

TABLE IX

Influence of desoxycorticosterone (DOC) (5 mg/100 g) on glycogen consumption of the isolated rat diaphragm

(from ref. 49)

(Each value is the mean of a series for numbers of animals used see Table VIII)

Additions to Ringer's Solution	Normal								Adrenalectomized							
	Glycogen (mg/100 g)						Work (cm.³/100 g muscle)		Glycogen (mg/100 g)						Work (cm³/100 g muscle)	
	Change				Consumed during work				Change				Consumed during work			
	Rest		Work						Rest		Work					
	—	DOC	—	DOC	—	DOC	—	DOC	—	DOC	—	DOC	—	DOC	—	DOC
None	− 85	− 45	−245	−140	160	95	27	26	− 40	− 40	−145	−117	105	77	22	39?
Glucose (100 mg/100 ml.) + insulin (1 unit)	+170	+ 15	− 90	−115	260	130	36	34	+ 90	+ 20	− 40	− 60	130	80	32	34
Glucose (400 mg/100 ml.) + insulin (1 unit)	+365	+165	+ 10	− 25	355	190	38	35	+290	+ 65	+ 80	− 10	210	75	28	30

adrenalectomized animal's diaphragm. It was confirmed by Bartlett and MacKay (1a) The glycogenolytic effect of corticosteroids is not present during anoxybiosis, that is if nitrogen is bubbled through the Ringer's solution in which the muscle is kept It is independent of the quantity of glycogen in the muscle at the beginning of the experiment (37)

Similar experiments were carried out with the anterior abdominal muscle of the mouse (Table X) The glycogen breakdown *occurring in the presence of* 0 05 mg /100 ml adrenaline is of the same order as that with 5 mg /100 ml desoxycorticosterone This quantity of adrenaline can completely inhibit the stimulation of glycogenesis by 1 unit of insulin, desoxycorticosterone was somewhat less inhibitory But desoxycorticosterone and adrenaline together had an overall glycogenolytic action as the last column of Table X shows Thus the apparent inhibition of glycogen production is actually due to glycogen breakdown

The glucose uptake by the surviving diaphragm is about *twice as much as corresponds to the glycogen produced* (33, 56) We have shown that only one-half of the glucose absorption is inhibited by desoxycorticosterone (40, 41)—as much as is used for glycogen production The other half of the glucose which is taken up is probably oxidized This inhibition of glucose uptake was studied recently by Candela *et al* using cortisone (6) It should be mentioned that in addition to inhibiting glycogenesis *desoxycorticosterone has also been shown to inhibit glucose* oxidation in brain (18), and kidney D-amino-acid oxidase (21)

Other corticosteroids, and also some additional steroids have a similar action The experiments of Wenner and Leupin (36, 84) are referred to in Table XI, desoxycorticosterone (5 mg per 100 ml) is the most active corticosteroid Cortisone and 11-desoxy-17 *hydroxycorticosterone have a similar inhibitory activity* and 17-hydroxycorticosterone (only with 10 mg per 100 ml) has a rather smaller one It is, however, doubtful how far such quantitative comparisons can be used owing to the differences in solubility of the corticosteroids and the variations in glycogen production of the different individual diaphragms *Nevertheless,* the order of activity seems to be as mentioned In Table XI the *decrease in the glycogen content* of the muscle is compared with

TABLE X

Influence of desoxycorticosterone (DOC) adrenaline and insulin on glycogen content of the anterior abdominal muscle of the mouse

Glycogen change after 60 minutes, expressed as percentage of initial value.

(Mean values of series) (from ref. 3)

Addition	None	DOC (5 mg/100 g)	Adrenaline (0.05 mg/100 g)	Insulin (1 unit)	Adrenaline (0.03 mg/100 g) + insulin (1 unit)	DOC (5 mg/100 g) + insulin (1 unit)	DOC (5 mg/100 g) + adrenaline (0.05 mg/100 g) + insulin (1 unit)
Without glucose	71	42	68				
With glucose	116		58	188	101		
				254	102		
				252		120	
				166	97		70

TABLE XI

Effect of corticosteroids on the glycogen content of the isolated rat diaphragm.

Number of animals	Added substance	Dose (mg / 100 ml)	Glycogen in diaphragm (mg /100 g)				Reference
			initial	after 90 min	with steroid after 90 min	Difference	
13	desoxycorticosterone	5	246	380	244	−136	84
12	cortisone	5	244	488	396	− 92	36
8	11-desoxy-17-hydroxycorticosterone	5	119	372	286	− 86	36
6	17-hydroxycorticosterone	10	97	196	144	− 52	36
16	corticosterone	5	339	433	367	− 66	84

TABLE XII

Glucose absorption in normal and adrenalectomized rats

(from ref 78)

Condition of animals	Number of animals	Days after adrenalectomy	Glucose absorbed after 60 min (mg)	t	p
Normal	9	55–58	441 ± 47.2		
Adrenalectomized	16		295 ± 19.9	2.86	<0.1
Normal	12	26–63	439 ± 25.9		
Adrenalectomized	12		238 ± 22.1	5.91	<0.01

the amount of glycogen produced in a parallel experiment without steroid

Strong inhibitory activity, similar to that with desoxycorticosterone, is shown by progesterone, 17-hydroxyprogesterone, pregnendiolene and pregnandiolone, and definite activity is also observed with testosterone and androstane, but none with dorsynolic acid oestradiol, aetiocholanic acid or oleic acid (84) *Stilboestrol has a strong inhibitory action on glycogen production* and Ingle has shown that it has also an intense diabetogenic action in the pancreatectomized adrenalectomized rat (26) testosterone is also active in this test Curiously enough, in Ingle's experiments this effect was only found in animals maintained with 'sub-diabetogenic amounts of extracts of the adrenal cortex and anterior pituitary

These facts argue against calling this inhibitory effect a 'non-specific steroid effect Its relation to the diabetogenic activity of steroids is obvious, but curiously enough desoxycorticosterone and progesterone are more active than 11-oxycorticosteroids The possibility that there is a competitive inhibition of a certain enzyme seems still open to discussion

This glycogenolytic effect of corticosteroids antagonizes the action of insulin in the muscle Such an antagonism has long been known Grattan and Jensen have shown that, in mice the hypoglycaemic convulsions following administration of insulin can be *inhibited with adrenal cortical extract* (20) and Peyser *found* that DOCA has the same effect (57) The usual explanation given for this effect was that the corticosteroids increased the amount of glycogen formed and thereby counteracted the hypoglycaemia produced by insulin But it is now uncertain whether such an explanation is valid

Long and his associates have shown that glycosuria is not observed in the pancreatectomized adrenalectomized rat, but that it reappears if corticosteroids are given (42 43) Thus lack of insulin leads to glycosuria but only if corticosteroids are present If insulin is given after corticosteroids, the glycosuria disappears

A third case of antagonism by insulin was observed by Ingle (24) Eviscerated rats were kept alive for a considerable time with continuous infusion of glucose and insulin Glucose was

used up at a constant rate If, however, adrenal cortical extract
that is,

etween
1-phos-
phate is produced from glycogen by phosphorylase The rate of breakdown of glycogen is lower after adrenalectomy, but can be restored by administration of corticosteroid (52) The latter *obviously acts on the glycogenolytic process,* while insulin increases glycogenesis

We are thus confronted with two experimental facts which seem to be completely contradictory In the adrenalectomized but otherwise intact animal, corticosteroids increase glycogen production, while in isolated muscle they increase glycogen breakdown The inhibition which was found (a) in the isolated diaphragm, *has its parallel* (b) in the intact animal, (c) in the eviscerated rat (d) in insulin-provoked hypoglycaemic convulsions, (e) in the excretion of sugar by adrenalectomized pancreatectomized animals, and (f) in muscle phosphorylase activity This leads to the conclusion that we are not dealing here with a nonspecific reaction corticosteroids seem to act primarily by increasing glycogen breakdown in the muscle Ingle came to similar conclusions (25) and Conn and Gordon observed an increase in the blood sugar level after administration of ACTH—'*a fact which lends strong support to the basic* thesis that the glycogenic steroids of the adrenal gland reduce the hypoglycaemic effect of insulin in the peripheral tissues' This means that the corticosteroids have an anti-insulin effect

The nature of this anti-insulin effect of corticosteroids must for the present remain unexplained Schur and his *school in*
ns-
ılin
ors
seem to agree that in liver slices insulin has an inhibitory action on glycogen production. Koepf *et al* have shown that liver slices from adrenalectomized animals produce 'total carbohydrate' from lactate and pyruvate (32) *The amount is increased* by treatment with adrenal cortical extract (without insulin) Chiu and Needham claimed similar results with glucose and

pyruvate as substrates (7) But Leupin was unable to reproduce this effect (36) Lubken recently found that desoxycorticosterone glucoside stimulated glycogen formation from DL-alanine and that this effect could be inhibited with insulin (45) (Desoxycorticosterone inhibited if added in advance)

EXPERIMENTS ON ABSORPTION

The experiments described above have led to the conception that the adrenal cortical steroids are concerned in the production of glucose in cell metabolism We must now discuss the question of which phase of cellular metabolism includes the reaction in which corticosteroids are involved

This problem was studied some years ago with the intestinal mucosa This is a single epithelial layer known to be selectively permeable to certain sugars The selective absorption of glucose and galactose is inhibited by phlorrhizin and other inhibitors of phosphorylating enzymes From mixtures of galactose and sorbose the former is promptly absorbed while the latter enters about five times more slowly (5) Atebrin and dinitrophenol poisons which uncouple oxidation and phosphorylation (44) are inactive Those sugars which are selectively absorbed were shown to be present in the intestinal mucosa as phosphoric esters (2 31 71) Meyerhof and Green showed that intestinal mucosal phosphatase transphosphorylates sugars producing hexose-1 or 6-phosphates (50) Hele found that the activity of intestinal hexokinase is related to the selective absorption of sugars (22) Selective absorption of glucose and galactose from the intestine is diminished in the adrenalectomized animal (9 17, 29 34 65 88)

The criticisms that the disturbance of selective absorption may be the result of changes in electrolyte metabolism or of hunger (1 47) and that animals maintained on sodium chloride give negative results (8) may be rejected in the light of recent experiments by Sailer and Verzár (78) Adrenalectomized rats were maintained on drinking water containing 1 per cent sodium chloride and were in a good state of health between twenty-six and sixty-three days after adrenalectomy Cori s method of assaying the amount of glucose absorbed was used The results of two independent series of experiments are given in Table XII

Thus adrenalectomized rats, kept alive for twenty-six to sixty-three days with 1 per cent sodium chloride in the drinking water, showed a decrease in selective glucose absorption of about 33–46 per cent This difference is significant

Disturbances in selective absorption are best demonstrated with mixtures of selected and non-selected sugars in equal amounts Methods for the differentiation of mixtures of glucose and xylose (66, 67) or of galactose and sorbose (5) were used The ratio of the amounts of selected and of non-selected sugar *in the intestine* of a normal animal is 1 to 3 or 4, after adrenalectomy it is 1 to 1 or 2 It was often not understood that it is not the absolute amount of absorption which is characteristically altered but the relative speeds of absorption of selectively and non-selectively absorbed sugars Table XIII gives the rates of absorption of glucose and xylose from mixtures of the two sugars

TABLE XIII

Effects of adrenalectomy and adrenal cortical extract on the relat ve rates of absorption of glucose and xylose from the intest ne

Animal	Ratio of the rates of glucose and xylose absorption			*Reference*
	Normal	Adrenalectomized	Cortin-treated	
rat	3 7 4 0	2 3	4.3	88
rat	3 03	0 75	—	34
cat	2 26–2 64	0 99–1 81	—	28
rat	5 2	2 4	4.9	117
frog	2 2 2 6	1 28	—	51

A similar problem was also attacked many years ago by Minibeck and Verzár (51) After an intravenous injection of equal amounts of different hexoses and pentoses, certain sugars such as glucose, galactose and fructose disappear quickly from the blood, while xylose, sorbose and ribose do not This effect is of the same order as with absorption from the intestine We injected 5 ml of various 20 per cent sugar solutions and compared the blood sugar values after 120 minutes (Table XIV) The experiments were performed on cats narcotized with 'Evipan'

Blood sugar curves are open to many different interpretations It must therefore be strongly emphasized that only the hyperglycaemic curves obtained in the same individual after intravenous injection of selectively and non-selectively absorbed sugars

should be compared If this is done non-selectively absorbed sugars are found to disappear much more slowly from the blood into the tissues than selectively absorbed sugars This difference vanishes in the adrenalectomized animal glucose, galactose and fructose are still present after 120 minutes at as high a concentration as mannose and xylose (Table XIV) This shows that adrenalectomy diminishes the selective absorption of sugars into the tissues It has to be emphasized again that not all sugars are taken up more slowly after adrenalectomy, but the difference in velocity with which the selectively and non-selectively absorbed sugars disappear is abolished after adrenalectomy

TABLE XIV

Sugar concentration in blood after intravenous injection of different hexoses and pentoses in normal and adrenalectomized cats

(from ref. 51)

Sugar injected (1 g)	Condition and number of animals		Increase in blood sugar concentration (mg /100 ml)		Type of absorption
			Maximal	After 120 min.	
Glucose	normal	(4)	93 to 118	−3 to +5	selective
Galactose	normal	(3)	81 to 116	+1 to +12	selective
Fructose	normal	(4)	83 to 114	−1 to +4	selective
Mannose	normal	(2)	101	+44 to +47	non-selective
Sorbose	normal	(2)	92	+33 to +34	non-selective
Xylose	normal	(3)	102 to 127	+42 to +63	non-selective
Glucose	adrenalect.	(4)	106 to 133	+50 to +77	selective
Galactose	adrenalect	(1)	125	+52	selective
Fructose	adrenalect.	(1)	144	+90	selective
Mannose	adrenalect.	(1)	110	+52	non-selective
Xylose	adrenalect	(1)	89	+36	non-selective

This finding was confirmed in man, by comparing patients with Addison's disease before and after administration of adrenal cortical extract (69) Treatment of Addison patients with adrenal cortical extract shortened the time span of the glucose curve, while the xylose curve remained the same Treatment had thus abolished the disturbance which was responsible for the slow uptake of glucose into the tissues in adrenal insufficiency

CONCLUSIONS

The conclusion which we draw from these detailed experiments is that the influence of corticosteroids on carbohydrate

metabolism is *not* mainly connected with glycogen formation, but with the *production of glucose* for the requirements of the cell. This glucose might come directly from the food, or from tissue glycogen, from protein or from fatty acids, according to availability. The point of action of corticosteroids is not the protein molecule or the deamination of amino-acids. It seems to be the reaction where glucose enters the metabolism of the cell, probably via certain phosphatases (74).

The seemingly contradictory facts of stimulation of glycogen production by corticosteroids in the whole animal and the anti-insulin effect, especially with isolated muscle, are no contradictions. If enough glucose is available for energy production, the rest is stored as glycogen. This view is supported by the observations on selective absorption of certain hexoses from the intestine and from the blood into the tissues, which (at any rate, for glucose and galactose) also requires the formation of a phosphorylated intermediate.

The availability of glucose in cell metabolism is the basic factor of excitability and energy production. It was the object of other work to show the intimate connection of carbohydrate metabolism with the metabolism of sodium and potassium (75). The polarization of the cell membranes in muscle and nerve is caused by the presence of a higher potassium concentration inside, and a higher sodium concentration outside, the cell. This polarization is changed during activation, and the restoration of this polarization is dependent on glucose metabolism. Inhibition of the latter makes the restoration of excitability and energy production impossible. It is at this basic cellular metabolic reaction that the corticosteroids intervene.

SUMMARY

1. In the adrenalectomized animal, the production of glycogen by liver and muscle can be restored by administration of 11-oxy-corticosteroids, 11-desoxycorticosterone also has an effect, but it acts more slowly.

2. The production of glycogen from glucose in starved adrenalectomized animals is also increased. Thus corticosteroids increase the availability of glucose for glycogenesis.

3. Doses of 11-desoxycorticosterone acetate (DOCA) large

enough to produce a state of unconsciousness, lead, on the contrary, to glycogen depletion in muscles and liver

[illegible]

gized by adrenaline, is also observed

5 The inhibition is strongest with 11-desoxycorticosterone, but cortisone and other corticosteroids also have a similar action This inhibition is explained as an 'anti-insulin' effect and correlated with other similar observations

6 The diaphragm from an adrenalectomized animal can produce glycogen, all phases of glycogen metabolism seem to be less active however

7 The isolated diaphragm is unable to produce glycogen from glucose-1-phosphate, whereas minced muscle does.

8 Selective absorption of glucose or galactose from the intestine is diminished in adrenalectomized animals, as can be shown by the use of mixtures of glucose and xylose

9 Selective absorption of glucose is also diminished in adrenalectomized animals (rats) kept alive for twenty-six to sixty-three days on drinking water containing 1 per cent sodium chloride

10 The selective absorption of glucose and other hexoses from the blood into the tissues is also diminished in the adrenalectomized animal.

11 The role of the adrenal cortical hormones in carbohydrate metabolism is that they make glucose available for cell metabolism This is the basic reaction, but by virtue of the interdependence of the metabolism of carbohydrate and of potassium and sodium, it is also responsible for the polarization of the cell membrane, probably because certain phosphorylating enzymes are influenced

REFERENCES

1 ALTHAUSEN T L., ANDERSON, E. M and STOCKHOLM, M., *Proc Soc exp Biol*, N Y, 40, 342 (1939)
1a BARTLETT, J A and MACKAY, E M *Proc Soc exp Biol*, N Y., 71, 493 (1949)
2 BECK, L. V, *J biol Chem* 143 403 (1942)
3 BOZOVIČ, L., LEUPIN, E. and VERZÁR, F, *Helv physiol acta* 7, 328 (1949)

4 BRITTON, S W, SILVETTE, H and KLINE, R, *Amer J Physiol*, 122, 446 (1938)
5 BRÜCKNER, J, *Helv physiol acta*, 9, 3 (1951)
6 CANDELA, J L R-, TAMARIT, J, CANDELA, R R- and CRISTENSEN-KÖNIG, W, *Arch Med exp*, 14, 7 (1951)
7 CHU, C Y and NEEDHAM, D M, *Biochem J*, 46, 114 (1950)
8 CLARK, W G and MACKAY, E M, *Amer J Physiol*, 137, 104 (1942)
9 CORI, C F, *J biol Chem*, 66, 691 (1925)
11 CSIK, L and LUDÁNY, G, *Pflug Arch ges Physiol*, 232, 187 (1933)
12 DERMAN, A, *Arch int Physiol*, 54, 314 (1946)
13 DOETSCH, R, *Helv chim acta*, 28, 31 (1945)
15 ENGEL, F L, SCHILLER, S and PENTZ, E J, *Endocrinology*, 44, 458, 476 (1949)
16 EVERSE, J W R and DE FREMERY, P, *Acta brev neerl Physiol*, 2, 152 (1932)
17 FITZGERALD, O, *Pflug Arch ges Physiol*, 241, 741, 242, 30 (1939)
18 GORDAN, G S and ELLIOTT, H M, *Endocrinology*, 41, 517 (1947)
20 GRATTAN, J F and JENSEN, H, *J biol Chem*, 135, 511 (1940)
21 HAYANO, M and DORFMAN, R I, *Ann N Y Acad Sci*, 54, 608 (1951)
22 HELE, M P, *Nature, Lond*, 166, 786 (1950)
23 INGLE, D J, *Amer J Physiol*, 116, 622 (1936)
24 INGLE, D J, *Publ Amer Ass Advanc Sci* (*The Chemistry and Physiology of Hormones*), p 83 (1944)
25 INGLE, D J, *Ann N Y Acad Sci*, 50, 576 (1949)
26 [illegible]
27 [illegible] 202 (1948)
28 ISSEKUTZ, B, Jr, LASZT, L and VERZÁR, F, *Pflug Arch ges Physiol*, 240, 612 (1938)
29 JUDOVITS, N and VERZAR, F [illegible], 292, 182 ([illegible]
30 KAHNT, F W and [illegible] *chim* [illegible] (1951)
31 KJERUL[illegible] K, *Acta* [illegible] 4, 249 [illegible]
32 K[illegible] H [illegible] and [illegible] *A*[illegible] 133, [illegible])
33 F[illegible] PARK, [illegible]
34 I[illegible], F, [illegible]

35 LEUPIN, E, *Biochem J*, 46, 567 (1950)
36 LEUPIN, E., unpublished experiments
[illegible]
40 LEUPIN, E and VERZÁR, F, *Biochem J*, 46, 562 (1950)
41 LEUPIN, E, VOGTLI, W and VERZÁR, F, *Helv physiol acta*, 7, C13 (1949)
42 LONG, C N H, KATZIN, B and FRY, E G, *Endocrinology*, 26, 309 (1940)
43 LONG, C N H and LUKENS, F D W, *J exp Med*, 63, 465 (1936)
44 LOOMIS, W F and LIPMANN, F, *J biol Chem*, 173, 807 (1948)
45 LUBKEN, W, *Arch exp Path Pharmak*, 214, 253 (1952)
47 MARAZZI, R, *Amer J Physiol*, 131, 36 (1940)
48 MENTHA, J and VOGTLI, W, *Helv physiol acta*, 5, C43 (1947)
49 MENTHA, J, VOGTLI, W and VERZÁR, F, *Helv physiol acta*, 6, 853 (1948)
50 MEYERHOF, O and GREEN, H J, *J biol Chem*, 178, 655 (1949)
51 MINIBECK, H and VERZÁR, F, *Helv med acta*, 5, Suppl 7 (1940)
52 MONTIGEL, C and VERZÁR, F, *Helv physiol acta*, 1, 137 (1943)
53 NISSIM, J A, *Nature, Lond*, 169, 504 (1952)
54 OLSON, R E, JACOBS, F A, RICHERT, D, THAYER, S A, KOPP, L J and WADE, N J, *Endocrinology*, 35, 430 (1944)
56 PERLMUTTER, M and GREEP, R O, *J biol Chem*, 174, 915 (1948)
57 PFYSER, E, *Verh schweiz Ver Physiol*, 28, 42 (1941)
58 REICHSTEIN, T, *Handb biol ArbMeth* 36, 1367 (1938)
59 REINECKE, R M and KENDALL, E C, *Endocrinology*, 32 505 (1943)
60 SASS-KORTSÁK, A, WANG, F C and VERZÁR, F, *Amer J Physiol*, 159, 256 (1949)
61 SASS-KORTSÁK, A, WANG, F C and VERZÁR, F, *Helv physiol acta*, 7, C18 (1949)
62 SCHUR, H, LOW, A and KREMA, A, *Wien Arch inn Med*, 24, 463, 25, 203 (1934)
63 SCHUR, H, LOW, A and KREMA, A, *Acta med scand*, 95, 167 (1938)
64 SELYE, H J, *J Pharmacol*, 71, 236 (1941)
65 SOULAIRAC, A, *C.R Soc Biol, Paris*, 142, 778 (1948)
66. STÄHELIN, D, Dissertation, Univ Basel (1946)
67 STÄHELIN, D and VOGTLI, W, *Helv physiol acta*, 6, 317 (1948)

68 STETTEN, D, Jr, *J biol Chem*, 159, 593 (1948)
69 THADDEA, S and SARKADY, L, *Schweiz med Wschr*, 73, 1331, 1400 (1943)
70 THOMSEN, V, *Acta med scand*, 96, Suppl XCI (1938)
71 VERZÁR, F, *Die Funktion der Nebennierenrinde*, Basel B Schwabe & Co (1939)
72 VERZÁR, F, *Schweiz med Wschr*, 80, 468 (1950)
73 VERZÁR, F, *Conferenza di Endocrinologia (2a Settimana di alta Cultura medica internationale)*, Firenze, p 1 (1950)
74 VERZÁR, F, in *Ciba Foundation Colloquia on Endocrinology*, vol. VI, ed WOLSTENHOLME, G E W, p 152, London Churchhill (1953)
75 VERZÁR F, in *The Suprarenal Cortex*, ed YOFFEY, J M, p 39 London Butterworth's Scientific Publications (1953).
76 VERZÁR, F, BUCHER, P, SOMOGYI, J C and WIRZ, H *Helv med acta*, 7, Suppl VI, 58 (1941)
77 VERZÁR, F, SAILER, E and RICHTERICH, R., *Helv physiol acta*, 10, 231 (1952)
78 [illegible] *Helv physiol acta* 10 217 (1952)
79 [illegible] 1937)
80 [illegible] 1950)
81 VERZÁR, F and WENNER, V, *Bull Soc Chim biol, Paris*, 29 304 (1947)
82 VERZÁR, F and WENNER, V, *Biochem J*, 42 35 (1948)
83 VERZÁR, F and WENNER V, *Biochem J*, 42, 42 (1948)
84 VERZÁR, F and WENNER, V, *Biochem J*, 42, 48 (1948)
85 VOGTLI, W, *Helv Physiol acta*, 8 74 (1950)
86 WANG, F C, *Nature, Lond*, 165 277 (1950)
87 WANG, F C and VERZÁR, F, *Amer J Physiol*, 159 263 (1949)
88 WILBRANDT, W and LENGYEL, L, *Biochem Z*, 267, 204 (1933)

DISCUSSION

LONG The values for the glycogen content of muscle given in your Tables VI and VII are approximately half of those we obtained The values found would seem to be related to the method used for estimation of the muscle glycogen, particularly the manner in which the muscle samples were obtained

VERZÁR The glycogen content of a muscle depends on its type we used the anterior rectus muscle of the hind limb The glycogen content of muscles, the diaphragm included, is influenced by the composition of the diet

YOUNG Did you examine the effect of a high protein diet on the glycogen content of the diaphragm?

VERZÁR This was not tested

LONG How were the animals killed?

VERZÁR They were killed by pithing and the muscle removed immediately (as described in ref 60) Narcosis gave no better results

LONG Under these conditions there is rigid contraction, and tetanic convulsions and liberation of epinephrine These will quickly reduce the glycogen content of the muscle to about one-half

RUSSELL It is well known that ether is a strong stimulator of epinephrine secretion

LONG It is very curious that in adrenalectomized depancreatized animals (which form a very sensitive preparation) 17-desoxycorticosterone is almost inactive in our experience and the 11-oxycorticosteroids are very active

VERZÁR We used 11-desoxycorticosterone and adrenalectomized animals with intact pancreases 11-Desoxycorticosterone acts if it is continuously administered after the adrenalectomy (ref 60)

LONG Three to four days was the period used in our experiments In one of Professor Verzár's experiments, 11-desoxy-17-hydroxycorticosterone was also found to be active As far as I know, no biological activity has been found with this substance by anybody else and it is thought to be devoid of all biological activity

CONN 400 mg per day of 11-desoxy-17-hydroxycorticosterone has been administered to a normal man without giving any evidence of a metabolic effect, except the excretion of large quantities of 17 ketosteroids when it is given orally

LONG I suggest that this may be a non-specific effect

VERZÁR 11-desoxy-17-hydroxycorticosterone had an effect similar to, but smaller than, 11-desoxycorticosterone on glycogen production by the isolated diaphragm The question of how far this inhibition could be a non-specific effect was discussed in my papers here and in London (ref 74)

RUSSELL Is it likely that any of the *in vitro* effects observed with the corticosteroids are the result of some non-specific, toxic action? Some indication might be obtained if the oxygen consumption were to be determined in the presence of these compounds Do these corticosteroids depress the metabolic rate?

VERZÁR An inhibition of oxygen uptake in brain tissue by desoxycorticosterone was seen by Grattan and Jensen (ref 20) and Hayano and Dorfman found that kidney D-amino-acid oxidase is inhibited by corticosteroids (ref 21)

RUSSELL Some of the effects of 11-desoxycorticosterone may be indirect, because very similar effects are obtained simply by giving salt to adrenalectomized rats In this case the fasting liver glycogen

THE ADRENALS AND GROWTH HORMONE DIABETES

By

C N H LONG

Department of Physiology, Yale University School of Medicine, New Haven, Connecticut, U S A

INTRODUCTION

It is an interesting but well-established fact that the anterior pituitary secretes two hormones, both of which are capable—when injected in sufficient amounts—of producing hyperglycaemia and glycosuria in normal animals These are the adrenocorticotrophic hormone (ACTH), and the growth hormone or a factor closely associated with the latter

Recent studies (12, 19, 20) suggest that the anterior lobe factor depressing the utilization of carbohydrate is not the same as that producing nitrogen retention and growth, and which is commonly termed the growth hormone It may be assumed that this 'carbohydrate hormone', if it exists, is not identical with the adrenocorticotrophic hormone and that it exerts its effects on the tissues without the mediation of another endocrine organ Until the possibility that the diabetogenic effects of 'growth hormone' are due to a separate pituitary principle has been more completely investigated, the term 'growth hormone' will be used to describe the highly active preparations obtained by the methods of Li *et al* (13) and Wilhelmi and his colleagues (22) Such preparations have, at least in experimental animals, both growth-promoting and diabetogenic activities

The purposes of this paper are (i) to discuss the relationship, if any, between the diabetogenic actions of these pituitary hormones, and (ii) to inquire whether the full diabetogenic potentialities of growth hormone preparations can be exerted in the absence of the adrenal glands

THE DIABETOGENIC EFFECT OF GROWTH HORMONE AND ACTH

The classical experiments of Houssay and his colleagues demonstrated that removal of the hypophysis greatly attenuated a total pancreatic diabetes These experiments were quickly followed by the observation that the injection of crude extracts

several laboratories have shown that highly purified growth hormone isolated from the anterior lobes of ox pituitaries by the methods of Li or Wilhelmi produces both temporary and permanent diabetes in cats and dogs (1, 3, 6)

It may therefore be concluded that the growth-promoting extracts of the anterior pituitary, when injected into normal fed animals, bring about a reduction in carbohydrate utilization

If similar pituitary extracts or growth hormone preparations are injected into *fasting* animals the blood glucose level falls. This paradoxical effect of apparently the same hormone in fed and fasted animals has not been satisfactorily explained It may

mixture under the action of growth hormone. This last effect would be of little consequence in fed animals, but in fasted

injection of these preparations is followed by a very rapid and frequently fatal hypoglycaemia.

The observations of Houssay that hypophysectomy attenuated a total pancreatic diabetes, whereas the injection of crude anterior pituitary extracts caused a temporary diabetes, immediately raised the question as to the nature of the pituitary factors that suppressed the utilization of carbohydrate The solution of this

the adrenal steroids have as the main feature of their action a capacity to accelerate the *catabolism of the tissue proteins* This makes available an increased quantity of carbohydrate precursors which are stored as liver glycogen Such an effect, while it obviously supplies increased quantities of carbohydrate for the support of the blood glucose level in times of need, is probably not the only benefit conferred by these hormones on the organism. The capacity to withstand stresses of all kinds, to effect an adequate separation of ions by the kidney tubules and to perform muscular work may also be related to the tissue protein changes that are influenced by these hormones The *nature of this relationship* is unknown, but we may anticipate that as our knowledge of the cellular processes of protein synthesis and degradation [illegible] of the adrenal [illegible] hat 'growth hormone' preparations exert their effects on carbohydrate and *protein metabolism in a different manner Their site of action* appears to lie in the extra-hepatic tissues, most probably in the skeletal muscles In this organ the growth hormone or a closely associated factor diminishes the utilization of carbohydrate, thus raising the blood glucose level and ultimately causing irreversible damage to the islets of Langerhans in some species Nevertheless, it must not be forgotten that—at least in the rat fed a high carbohydrate diet—*the adrenal cortical steroids* or *ACTH may also* cause a diminution in glucose utilization by the peripheral tissues Whether their site of action in these tissues is identical with that of growth hormone is not known

THE EFFECT OF GROWTH HORMONE ON CARBOHYDRATE METABOLISM IN THE ABSENCE OF THE ADRENALS

It was observed by Long and Lukens that the 'adrenocorticotrophic-prolactin' fraction prepared by Collip increased the glycosuria of hypophysectomized depancreatized cats but was ineffective in adrenalectomized depancreatized animals (15) Long *et al* found that a saline extract of anterior pituitary which increased the glycosuria of partially depancreatized rats frequently *failed to do so after total adrenalectomy* (14) This could not be

attributed to an insufficiency of adrenal cortical hormones since the animals received adequate amounts of sodium salts and were apparently in good health. It was also interesting that there were abrupt gains in body weight during the period of treatment both in the presence and absence of the adrenals

However when the adrenalectomized depancreatized rats were given a constant amount of adrenal cortical extract daily the injection of the same saline extract of anterior pituitary caused still greater glycosuria Houssay and Biasotti also found that if adrenalectomized depancreatized dogs were given adequate daily amounts of adrenal cortical extract the injection of anterior pituitary extracts produced a marked elevation of the blood glucose level (7)

These experiments lead to two conclusions

(i) That crude anterior pituitary extracts contain a factor other than ACTH which can cause hyperglycaemia and glycosuria and (ii) that the full activity of this second (extra adrenal) factor is not manifested unless a certain minimal quantity of adrenal cortical hormone is present in the body The evidence cited above indicates that the second potentially diabetogenic factor in anterior pituitary extracts is growth hormone or some other agent that remains closely associated with it in the extraction methods used.

There are other lines of evidence that support the above conclusions Russell has reported that the capacity of anterior pituitary extracts to lower the respiratory quotient and increase muscle glycogen deposition (the glycostatic effect) in normal rats is abolished by adrenalectomy but reappears when small amounts of adrenal cortical hormone are given along with the pituitary extract (21) It has subsequently been shown that highly purified growth hormone preparations also have a glycostatic effect (8)

There are other examples in the literature of this necessity for the presence in the body of minimal amounts of adrenal cortical hormones if the full activity of other hormones is to be expressed. Among them is the demonstration by Ingle that the inhibition of hair growth by oestrogens in the rat does not occur in adrenalectomized animals (10) However the effect is restored if small but constant quantities of adrenal cortical hormone are given Doubtless there are many situations in which the organs and

tissues of the body do not respond in a normal manner if deprived of adrenal cortical hormones

It is also known that a deficiency of other factors essential for growth, such as vitamins and minerals, will also reduce or abolish the usual response to growth hormone An example of this is the failure of rats deficient in vitamin A to respond to growth hormone (17)

The observations on the failure of totally adrenalectomized animals to respond to growth hormone preparations has its parallel in the *in vitro* studies by Park and Krahl (12, 18) These investigators found that the prior administration of growth hormone preparations only slightly decreased the glucose uptake of the isolated diaphragm of hypophysectomized depancreatized rats However, a marked depression of glucose uptake was

The failure of animals deprived of adrenal cortical hormones to develop hyperglycaemia and glycosuria when injected with growth hormone preparations does not necessarily signify a close functional synergism between these two hormones It may only indicate the requirement of many organs and tissues for adrenal cortical hormone in order to carry out their various functions

The experiments of Engel and his colleagues with normal rats *do suggest however that in this species, which is notoriously* resistant to the diabetogenic action of growth hormone, an increased rate of secretion by the adrenal cortex must be established before diabetes can occur (4) They found that administra-

ACTH ... rats force-fed

...oduced glyco-

...ier, glycosuria

and hyperglycaemia were always observed The reasons for the synergism found in the rat between these hormones are not known Engel and his colleagues, following the argument of *Young, suggest that it may be related to the restriction of protein* storage usually occurring under the action of growth hormone, that is imposed by a high level of adrenal cortical secretion (4)

CONCLUSIONS

1. There is little doubt that ACTH exerts its effects on carbohydrate and protein metabolism by increasing the rate of secretion of adrenal cortical steroids.

2. The adrenal steroids that influence carbohydrate and protein metabolism are of the 11-oxygenated type such as cortisone and 17-hydroxycorticosterone

[illegible]

4. In animals fed diets containing carbohydrate, the diabetogenic properties of these steroids vary from species to species In the rat, marked glycosuria, far exceeding any that could be derived from tissue protein breakdown, has been found to follow their injection In the dog, ACTH elicits only a mild diabetes (1) In man, diabetes of a moderate degree may be induced by both cortisone and ACTH.

5. The anterior pituitary secretes another hormone or hormones that have a marked effect on carbohydrate and protein metabolism. The growth-promoting and protein-sparing effects of extracts of this gland are to be attributed to growth hormone. It is still an open question whether the depression of carbohydrate utilization produced by anterior lobe extracts is a property of growth hormone or is due to another agent which remains closely associated with it in the present methods of extraction. The presence of this second agent in growth hormone preparations may well explain the paradoxical effects of such preparations on the carbohydrate metabolism of fed and fasted animals.

6 The preparations of growth hormone used up to the present time initially decrease the nitrogen excretion of fed or fasted normal, adrenalectomized or hypophysectomized animals. This clearly distinguishes the effect of these preparations from that of adrenal cortical steroids, which always augment nitrogen excretion if given in sufficient amount.

7. These preparations cause hyperglycaemia and glycosuria

[illegible]

growth-promoting material may be prepared that is relatively deficient in diabetogenic activity in the dog (19)

8 In fasting animals, the same preparations will cause hypoglycaemia—a paradoxical effect, which may be due to the preponderance of the nitrogen-retaining effect in these animals, or to other causes

9 The absence of adrenal cortical steroids modifies the diabetogenic effect of growth hormone preparations This may be restored by the administration of adrenal cortical hormones in amounts that by themselves do not significantly disturb the state of equilibrium of carbohydrate metabolism.

10 *There is reason to believe that the point of action of* adrenal cortical steroids is on some phase of protein metabolism by which the supply of carbohydrate precursors is increased. The main effect of growth hormone preparations on carbohydrate metabolism appears to be the suppression of carbohydrate utilization

REFERENCES

1 Campbell, J, Davidson, I W F, Snair, W D and Lei, H P, *Endocrinology*, 46, 273 (1950)
2 Conn, J W, Louis, L H and Johnston, M W, *Proc Amer Diabetes Ass*, 8, 213 (1948)
3 Cotes, P M, Reid, E and Young, F G, *Nature, Lond*, 164 209 (1949)
4 Engel, F L, Viau, A, Coggins, W and Lynn, W S, *Endocrinology*, 50, 100 (1952)
5 Harrison, H C and Long, C N H, *Endocrinology*, 26, 971 (1940)
6 Houssay, B A and Anderson, E, *Endocrinology*, 45, 627 (1949)
7 Houssay, B A and Biasotti, A, *Rev Soc argent Biol*, 14, 308 (1938)
8 Illingworth, B and Russell, J A, *Endocrinology*, 48, 423 (1951)
9 Ingle, D J, *Endocrinology*, 29, 649 (1942)
10 Ingle, D J, *Recent Progr Hormone Res*, 6, 159 (1951)
11 Ingle, D J, Li, C H and Evans, H M, *Endocrinology*, 39, 32 (1946)
12 Krahl, M E, *Ann N Y Acad Sci*, 54, 649 (1951)
13 Li, C H, Evans, H M and Simpson, M E, *J biol Chem*, 159, 353 (1945)

14 LONG, C. N. H., KATZIN, B. and FRY, E. C., *Endocrinology*, 26, 309 (1943)
15 LONG, C. N. H. and LUKENS, F. D. W., *J. exp. Med.*, 63, 465 (1936)
16 LUKENS, F. D. W. and DOHAN, F. C., *Endocrinology*, 22, 51 (1938)
17 MARGITAY-BECHT, E. and WALLNER, E., *Z. Vitaminforsch.*, 6, 119 (1937)
18 PARK, C. R. and KRAHL, M. E., *J. biol. Chem.*, 181, 247 (1949)
19 RABEN, M. S., WESTERMEYER, V. W. and LEAF, A., *J. clin. Invest.*, 31, 655 (1952)
20 RECANT, L., *J. clin. Invest.*, 31, 656 (1952)
21 RUSSELL, J. A., *Amer. J. Physiol.*, 128, 552 (1940)
22 WILHELMI, A. E., FISHMAN, J. B. and RUSSELL, J. A., *J. biol. Chem.*, 176, 735 (1945)

DISCUSSION

CARL CORI: I should like to describe some experiments in which Park, in our laboratory, noted an initial hypoglycaemic action of growth hormone in the eviscerated rat. The diaphragm after isolation showed an increased glucose uptake 1–2 hours after injection of the growth hormone. This was followed at 3 hours by an inhibition of the glucose uptake; this inhibitory effect was stronger with crude extracts than with pure growth hormone. When microgram amounts of growth hormone (less than sufficient to give maximal growth) were injected into hypophysectomized rats the glucose uptake was strongly inhibited. The phenomenon may be due to growth hormone itself or possibly to some metabolic derivative of growth hormone.

This effect is consistent with other observations by Park and Bornstein such as the following: (i) the serum of alloxan diabetic rats inhibits the glucose uptake of the normal diaphragm but this inhibitory effect is not obtained with serum taken from diabetic rats which have undergone adrenale[illegible]
substance reappears [illegible]
ectomized diabetic [illegible]
It seems therefore that there are at least two pituitary factors (i) growth hormone or a closely related substance, which affects the glucose uptake, and (ii) a substance which does not affect the glucose uptake, which is not antagonized by insulin and which depresses the respiratory quotient. The site of action of the two substances is different—according to Recant the depressant action on the respira-

tory quotient is located between the hexosemonophosphate and pyruvate stages of glucose degradation

YOUNG Was the pancreas absent in all these experiments

CARL CORI The animals were eviscerated and also given a standard dose of glucose They were then injected with growth hormone and the fall in the blood glucose level occurred *immediately* No pancreatic [illegible]

[illegible] ent upon

CARL CORI There is very little insulin in an eviscerated rat and there is no evidence that growth hormone enhances the action of the insulin

LONG Dr Wilhelmi s preparations of growth hormone very rapidly cause hypoglycaemia—great enough to kill an adrenalectomized animal

CARL CORI De Bodo found that when growth hormone was injected into an acutely depancreatized dog it produced an immediate fall in the blood sugar level

BEST There may be an increased liberation of insulin whether or not the pancreas is present

RUSSELL When pituitary extract consisting mainly of growth hormone was given to rats approximately one hour before evisceration the rate of glucose administration necessary to maintain the blood sugar level had to be reduced rather than increased

CARL CORI In our case the animals were injected immediately before evisceration.

GERT [illegible]

phase

WILI [illegible]

primary effects of growth hormone are on the peripheral tissues rather than on the liver

LONG There is no effect on the liver glycogen level and the degree of glycosuria is not related to the amount of nitrogen excreted (which usually decreases) A diversion of glycogen probably occurs in the peripheral tissues

WILHELMI There is evidence that both growth hormone and the adrenal cortical hormones may be involved in the synthesis of fat from carbohydrate in the liver

LUKENS In the experiments of Brady and Gurin in which growth hormone was given to two Houssay animals fat synthesis in the liver was completely blocked (These experiments were carried out by using [^{14}C]acetate) I believe this agrees with the results of Folley s experiments with mammary gland slices

LONG Was fat synthesis blocked also by ACTH?

LUKENS This was a less satisfactory study, though cortisone greatly reduced the rate of fat synthesis.

CAMPBELL: The results of one of our experiments are perhaps relevant to this question about the site of action of growth hormone. Intact dogs were treated with enough growth hormone to provoke diabetes. An increase in the weight and fat content relative to body weight occurred There was a marked decrease in the amount of insulin extractable from the pancreas, that is, a relative insulin deficiency was produced This is a rather complicated situation but the results suggest that growth hormone influences the liver

LONG You would therefore expect to get an inhibition of utilization of glucose by the diaphragm

CAMPBELL: There is further evidence that growth hormone has an [illegible] stimulated protein synthesis in the livers of these animals.

[illegible] hypoglycaemic action

LONG In our experiments, the hypoglycaemia persisted for a [illegible] long time

RUSSELL I can confirm that this happens

LAZAROW In view of Engel's finding that ACTH plus grow[illegible] more produces diabetes in the intact rat, why does crude [illegible] extract not give rise to diabetes in the rat?

LONG We used ox pituitary extracts which contain [illegible] ACTH

YOUNG Our crude alkaline extracts of ox anterior [illegible] are extremely poor in ACTH as determined by the Sa[illegible] think that there are probably very active proteolytic enzy[illegible] extracts which destroy ACTH

LONG Pig glands would probably have had a diabe[illegible] in Young's and Houssay's experiments

CAMPBELL Does the diabetogenic effect of growth [illegible] dogs act via the adrenals? This could be studied using [illegible] mized dog in which the pancreas has been left intact.

LONG This was done by Houssay, but he did not use purified growth hormone

YOUNG Lockett, Reid and I (unpublished) found purified growth hormone to be diabetogenic in adrenalectomized dogs maintained on adrenal extract.

LONG Houssay has done similar experiments with the toad.

THE STRUCTURE OF GLYCOGEN AND ENZYME PATTERNS IN GLYCOGEN STORAGE DISEASE

By

GERTY T. CORI

Department of Biological Chemistry, Washington University School of Medicine, St Louis, Missouri U S A

ENZYMIC DETERMINATION OF THE STRUCTURE OF POLYSACCHARIDES

Our knowledge of the structure, enzymic synthesis and degradation of glycogen and starch has been greatly advanced in the last decade These polysaccharides are perhaps the only natural polymers the fine structure of which is now fairly well understood. They are also the simplest, being composed exclusively of glucose residues The analysis of the structure of glycogen and starch has been made possible because the mechanism of action of the three enzymes which synthesize it and break it down is known Phosphorylase acts both in synthesis and breakdown, whereas amylo-α-1 6-glucosidase acts only in breakdown and the α-1 4'→α-1 6-transglucosidase only in synthesis

Phosphorylase is specific for the synthesis and degradation of the α-1 4' linkage between glucose residues In the presence of inorganic phosphate, glucose units from the outer chains of the polysaccharide are split off as glucose-1-phosphate When the enzyme reaches a branch point, where one glucose residue is united to three others instead of to two (the third one being in α-1 6' linkage), its activity is stopped, the residual polysaccharide



product is a dextrin in which what was the outermost tier of inner branches has become outer branches and which can now be further degraded by phosphorylase This stepwise degradation can be repeated several times and in some experiments 90 per cent of the polysaccharide has been thus degraded *in four or five two-*

LONG This was done by Houssay, but he did not use purified growth hormone

YOUNG Lockett, Reid and I (unpublished) found purified growth hormone to be diabetogenic in adrenalectomized dogs maintained on adrenal extract

LONG Houssay has done similar experiments with the toad.

THE STRUCTURE OF GLYCOGEN AND ENZYME PATTERNS IN GLYCOGEN STORAGE DISEASE

By

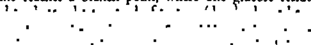
GERTY T CORI

Department of Biological Chemistry, Washington University School of Medicine, St Louis, Missouri, U S.A

ENZYMIC DETERMINATION OF THE STRUCTURE OF POLYSACCHARIDES

Our knowledge of the structure, enzymic synthesis and degradation of glycogen and starch has been greatly advanced in the last decade These polysaccharides are perhaps the only natural polymers the fine structure of which is now fairly well understood They are also the simplest, being composed exclusively of glucose residues The analysis of the structure of glycogen and starch has been made possible because the mechanism of action of the three enzymes which synthesize it and break it down is known Phosphorylase acts both in synthesis and breakdown, whereas amylo-α-1 6-glucosidase acts only in breakdown and the α-1,4'→α-1 6-transglucosidase only in synthesis.

Phosphorylase is specific for the synthesis and degradation of the α-1 4' linkage between glucose residues In the presence of inorganic phosphate, glucose units from the outer chains of the polysaccharide are split off as glucose-1-phosphate When the enzyme reaches a branch point, where one glucose residue is



smaller than the parent molecule. In this limit dextrin the glucose residue in 1 6 linkage has been exposed and thus dextrin is the substrate of amylo-α-1 6'-glucosidase While this enzyme does not act at all on native glycogen, it can now split the 1 6 linkage, giving free glucose as one reaction product. The other reaction product is a dextrin in which what was the outermost tier of inner branches has become outer branches and which can now be further degraded by phosphorylase This stepwise degradation can be repeated several times and in some experiments 90 per cent of the polysaccharide has been thus degraded in four or five two-

LONG Tl[illegible]
hormone [illegible]
YOUNG [illegible]
hormone [illegible]
on adrena[illegible]
LONG [illegible]

TABLE I

S[illegible]ral analy[illegible] [illegible]
([illegible])

[illegible] groups ([illegible] assay) (per cent)	[illegible]	Glycogen content of tissue (per cent)	Remarks
[illegible]	[illegible]	4.6	autopsy
8.4	[illegible]	0.55	autopsy
9.[illegible]	[illegible]		
[illegible]	[illegible]	0.96	biopsy
8	[illegible]	0.55	biopsy

TABLE II

[illegible] Gierke's disease.
([illegible] ref. 11)

[illegible]	[illegible] groups ([illegible] assay) (per cent)	Degraded by phosphorylase (per cent)	Glycogen content of tissue (per cent)	Remarks
[illegible] [illegible]	8.[illegible] 8.4	35.4 35.4	11.0 0.1	autopsy
[illegible] [illegible]	[illegible] [illegible]	35.4 [illegible]	2. 3	
Liver	[illegible]	4[illegible]		
Liver		[illegible]		
Liver		[illegible]		
Liver		[illegible]		
Liver				
Liver Muscle				
Liver Muscle				
Liver				

or biopsy There is considerable variation in end group percentage and even more in the lengths of the outer branches, as [illegible] Similar [illegible] shows the [illegible] e 9 were confined to the inner organs, whereas in case 9 both liver and muscles were used In all but the two last cases the glycogens were within the normal range

The two fatal cases of the disease are listed first In case 2 the liver glycogen content was not very high, probably because of the very high fat content (23 per cent) (Note the very high kidney glycogen content.) Cases 3–8 were older children, in fact case 7 was 19 years old In all of them hepatomegaly and a [illegible] ie, [illegible] of [illegible]

The glycogens in cases 9 and 10 had a structure never encountered in other men or animals and must be classed as abnormal Case 9 had glycogen storage in both liver and muscles The child is 12 years old and, but for a large liver, fairly well developed and healthy The outer chains in the liver glycogen were very short, so that the end group percentage of the whole molecule was very high This was even more marked in the muscle glycogen, the polysaccharide approaching a phosphorylase limit dextrin in structure and having barely any outer branches

The liver glycogen of case 10 was even more abnormal, but in the opposite direction It should really be classed as an amylopectin and not a glycogen The low percentage of end groups and the long outer chains were characteristic of amylopectins As was to be expected from structural analysis, this 'glycogen' gave a purplish colour with iodine It was only sparingly soluble in cold water and retrograded on freezing An X-ray diagram showed the crystallinity characteristic of amylopectins, true glycogens are amorphous

ENZYME PATTERNS IN VON GIERKE'S DISEASE

One might suspect that the muscle and liver of case 9 were deficient in the debranching enzyme (amylo-1 6-glucosidase)

TABLE I

Str ctural analys s of h man glycogens
(from ref 11)

Source of glycogen	End groups (enzymic assay) (per cent)	Degraded by phosphorylase (per cent)	Glycogen content of tissue (per cent)	Remarks
Infant liver (P J)	7 1	31 9	4 6	autopsy
Infant liver (R.O)	8 4	41 0	0 56	autopsy
Leg muscle	8 0	34 6		
Pectoralis muscle (one patient)	7 2	32 7	0 96	b opsy
Pectoralis muscle (pooled from two patients)	8 1	25 0	0.56	b opsy

TABLE II

Structure of glycogen in von G erke s disease
(from ref 11)

Case no	Source of glycogen	End groups (enzymic assay) (per cent)	Degraded by phosphorylase (per cent)	Glycogen content of tissue (per cent)	Remarks
1 (P S)	Liver Muscle	8 0 8 4	36 4 38 4	11 0 0 1	autopsy
2 (M A S)	Liver K dney	9 2 9 5	36 4 32 8	2 8 3 8	
3 (R D W)	Liver	7 4	34 6	10 2	b opsy
4 (Dennis B)	Liver	7 6	42 0	14.2	
5 (Daniel B)	Liver	7 6	41 0	12 7	
6 (T S)	L ver	8 9	32 8		„
7 (Doris B)	Liver	8 2	40 6	10 3	
8 (S S)	Liver Muscle	8 9 7 6	33 3 34.8	12 8 2 1	
9 (C H)	Liver *Muscle*	10 8 *13 1*	12 2 2 6	8 7 4 6	
10. (P.B)	Liver	4 7	50 5	2 9	autopsy

or biopsy There is considerable variation in end group percentage and even more in the lengths of the outer branches as [illegible] confined to the inner organs, whereas in case 9 both liver and muscles were used In all but the two last cases the glycogens were within the normal range

The two fatal cases of the disease are listed first In case 2 the liver glycogen content was not very high, probably because of the very high fat content (23 per cent) (Note the very high kidney glycogen content) Cases 3–8 were older children, in fact case 7 was 19 years old In all of them hepatomegaly and a low fasting blood sugar level were the main symptoms Some, but not all, had flat blood sugar curves after administration of epinephrine and a slightly diabetic response to a glucose meal

The glycogens in cases 9 and 10 had a structure never encountered in other men or animals and must be classed as abnormal Case 9 had glycogen storage in both liver and muscles The child is 12 years old and, but for a large liver, fairly well developed and healthy The outer chains in the liver glycogen were very short, so that the end group percentage of the whole molecule was very high This was even more marked in the muscle glycogen the polysaccharide approaching a phosphorylase limit dextrin in structure and having barely any outer branches

The liver glycogen of case 10 was even more abnormal, but in the opposite direction It should really be classed as an amylopectin and not a glycogen The low percentage of end groups and the long outer chains were characteristic of amylopectins As was to be expected from structural analysis, this 'glycogen' gave a purplish colour with iodine It was only sparingly soluble in cold water and retrograded on freezing An X-ray diagram showed the crystallinity characteristic of amylopectins, true glycogens are amorphous.

ENZYME PATTERNS IN VON GIERKE'S DISEASE

One might suspect that the muscle and liver of case 9 were deficient in the debranching enzyme (amylo-1 6 -glucosidase)

If such were the case, only the outer branches of the glycogens

tunately the small size of the biopsy samples of tissues did not permit an investigation of the debranching enzyme to be made

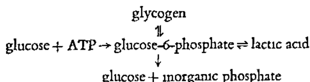
In case 10 a deficiency of the branching enzyme (transgluco-

which may not have been previously analysed, is attested by the *death of a sibling showing identical symptoms* Unfortunately no tissue was available for enzyme studies, nor could any other *than liver glycogen be analysed*

Enzymic studies were carried out in six cases of storage disease and in a number of human control cases The sequence of synthetic reactions by which glycogen is formed is obviously not disturbed, they are

glucose→glucose-6-phosphate⇌glucose-1-phosphate⇌glycogen

Glucose-6-phosphate can also be formed from non-carbohydrate sources, such as lactic acid, glycerol or amino-acids Blood sugar formation in the liver can be illustrated thus



If the phosphatase which splits glucose-6-phosphate were lacking, the overall equilibrium of these reactions would favour glycogen deposition, and both glycogen and non-carbohydrate sources would be unavailable for blood sugar formation It was therefore decided to study the specific phosphatases in von Gierke's disease

Livers obtained at autopsy were frozen within four hours and those obtained at biopsy were frozen in the operating room. *The frozen material was homogenized* with water and strained through gauze Each homogenate (equivalent to 4–33 mg liver)

was incubated in buffered solution for 1 hour at 30°C in the presence and absence of glucose-6-phosphate. After deproteinization, inorganic phosphate was determined in an aliquot of the filtrate. Values obtained after incubation without ester were subtracted from those obtained with ester.

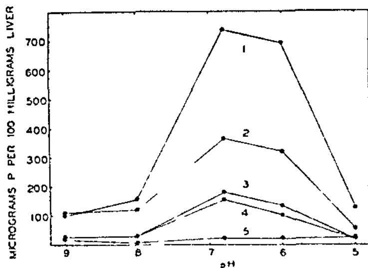


FIG. 2

Action of liver phosphatases on glucose-6-phosphate at different pH values.
Liver samples 1 normal, 2 cirrhotic, 3, 4, 5 from von Gierke cases. Samples 1, 2 and 5 were autopsy and 3 and 4 biopsy specimens. (From ref. 6)

Fig. 2 shows the phosphatase activity at different pH values. It will be seen that the pH optimum of the phosphatase is between 6 and 7, as had been previously found in animal livers. In a cirrhotic liver the activity was considerably weaker than in the normal liver. The three homogenates from the von Gierke cases had still lower activity, in fact in the fatal case 5 there was almost no phosphatase activity at any pH. At pH values of 9, 8 and 5 the specific phosphatase may still have had some activity in cases 1 and 2, in addition to the unspecific alkaline and acid phosphatases. When tested with glycerophosphate as substrate there was no marked difference between the homogenates 1

(normal) and 5 (von Gierke) Cases 3 and 4 were older children with mild manifestations of the disease

In addition the activity of glucose-6-phosphatase at pH 6 8 was studied in three biopsy samples, two from diseased and one from a normal liver The values obtained are shown in Table III

There was one more fatal case of von Gierke's disease (M A S) from whom liver was also obtained at biopsy In both biopsy and autopsy samples there was practically no phosphatase activity For two additional mild von Gierke cases the activity was in the normal range in case D B and in the range found in other liver diseases in case S S In the latter individual epinephrine produced a small but definite rise in the blood sugar level whereas in case D B there was no rise at all

The possibility that an inhibitor of the phosphatase was present

TABLE III

Glucose-6-phosphatase activity in liver homogenates at pH 6 8
(from ref. 6)

Case	No of expts	μg P liberated in 1 hour at 30 C by 100 mg liver	Remarks
K.M	2	73	10 years old, abdominal tumour
D S	4	327	8 years old, liver cirrhosis
C.S	2	429	1½ months old normal liver
M A S	2	32	von Gierke's disease
P S *	4	23	
Dennis B	2	155	
C H.	3	180	
S S	3	282	
Daniel B	2	404	
D S		312	
M A S *		15	
D S + M A S		294	

* Autopsy specimen

Boiled juice of normal liver did not increase the phosphatase activity in these two livers, showing that the lack of activity was not due to the absence of cofactors

Phosphoglucomutase activity was estimated in one of the fatal cases and in several controls Glucose-1-phosphate was

added instead of glucose-6-phosphate and the amount which disappeared accounted for as inorganic phosphate and glucose-6-phosphate The amounts of inorganic phosphate formed were almost exactly the same as when glucose-6-phosphate was the substrate, but in the normal livers only about one-quarter as much glucose-6-phosphate accumulated as in the von Gierke liver showing that the mutase step was rapid enough in the von Gierke liver to provide plenty of substrate for the phosphatase

The abnormal glycogen storage in the two fatal cases might be explained by the absence of glucose-6-phosphatase The genetically determined absence of this enzyme would be the primary cause of the disease A large number of secondary, tertiary etc. symptoms would follow

The mild cases are very difficult to evaluate. In two of these the phosphatase activity was at the level found in other liver diseases, or even near normal. It is impossible to estimate to what levels glucose-6-phosphatase activity has to be reduced before glycogen begins to accumulate, because this will be relative to the rates at which glycogen is formed from glucose and other sources. The disparity between these rates might be much greater in von Gierke's disease than, for instance, in cirrhosis where all enzymes including hexokinase, might be expected to have low activity

REFERENCES

1 Cori, C. F and Cori G T., *J b l. Chem.*, 158 341 (1945)
2 Cori C. F, Cori, G T and Green A A., *J b l. Chem.*, 151 39 (1943)
3 Cori, G T *J b l. Chem.*, 158 333 (1945)
4 Cori, G T and Cori, C. F., *J b l Chem.*, 151 57 (1943)
5 Cori G T and Cori, C F, *J b l Chem.*, 158 321 (1945)
6 Cori G T and Cori, C. F *J b l. Chem*, 199 661 (1952)
7 Cori G. T and Green A. A *J b l Chem*, 151 31 (1943)
8 Cori G T and Larner J., *J b l Chem* 188 17 (1951)
9 Green A. A., *J b l Chem* 158 315 (1945)
10 Green A. A and Cori G T., *J b l. Chem.*, 151 21 (1943)
11 Illingworth, B and Cori G T., *J b l. Chem.*, 199 653 (1952)
12 Illingworth, B., Larner J and Cori G T., *J b l. Chem*, 199 631 (1952)
13 Larner J, Illingworth B., Cori G T and Cori, C. F., *J b l. Chem* 199 641 (1952)

(normal) and 5 (von Gierke) Cases 3 and 4 were older children with mild manifestations of the disease

In addition, the activity of glucose-6-phosphatase at pH 6 8 was studied in three biopsy samples, two from diseased and one from a normal liver. The values obtained are shown in Table III

There was one more fatal case of von Gierke's disease (M A S) from whom liver was also obtained at biopsy. In both biopsy and autopsy samples there was practically no phosphatase activity. For two additional mild von Gierke cases the activity was in the normal range in case D B. and in the range found in other liver diseases in case S S In the latter individual, epinephrine produced a small but definite rise in the blood sugar level, whereas in case D B there was no rise at all

The possibility that an inhibitor of the phosphatase was present was tested by mixing the homogenate from a fatal case, in which the enzymic activity was near zero, with the homogenate from a normal liver No significant inhibition of the normal phosphatase activity resulted, as shown in the last three lines in Table III

TABLE III

Glucose-6-phosphatase activity in liver homogenates at pH 6 8
(from ref 6)

Case	No of expts	μg P liberated in 1 hour at 30°C by 100 mg liver	Remarks
K.M	2	273	10 years old, abdominal tumour
D S	4	327	8 years old, liver cirrhosis
C S	2	429	1½ months old, normal liver
M.A S	2	22	von Gierke's disease
P S *	4	21	" " "
Dennis B	2	155	" "
C H.	3	180	" "
S S	3	282	" " "
Daniel B	2	404	" "
D S		312	
M A.S *		15	
D S + M A.S		294	

* Autopsy specimen

Boiled juice of normal liver did not increase the phosphatase activity in these two livers, showing that the lack of activity was not due to the absence of cofactors

Phosphoglucomutase activity was estimated in one of the fatal cases and in several controls Glucose-1-phosphate was

almost exactly the same as when glucose-6-phosphate was the substrate, but in the normal livers only about one-quarter as much glucose-6-phosphate accumulated as in the von Gierke liver, showing that the mutase step was rapid enough in the von Gierke liver to provide plenty of substrate for the phosphatase

The abnormal glycogen storage in the two fatal cases might be explained by the absence of glucose-6-phosphatase The genetically determined absence of this enzyme would be the primary cause of the disease A large number of secondary, tertiary etc symptoms would follow

The mild cases are very difficult to evaluate In two of these the phosphatase activity was at the level found in other liver diseases, or even near normal It is impossible to estimate to what levels glucose-6-phosphatase activity has to be reduced before glycogen begins to accumulate, because this will be relative to the rates at which glycogen is formed from glucose and other sources The disparity between these rates might be much greater in von Gierke's disease than, for instance, in cirrhosis, where all enzymes, including hexokinase, might be expected to have low activity

REFERENCES

1 CORI, C F and CORI, G T, *J biol Chem*, 158, 341 (1945)
2 CORI, C F, CORI, G T and GREEN A A, *J biol Chem*, 151, 39 (1943)
3 CORI, G T, *J biol Chem*, 158, [illegible]
4 C
5 C
6 C
7 CORI, G T and GREEN, A A, *J biol Chem*, 151, 31 (1943)
8. CORI, G T and LARNER J *J biol Chem* 188 17 (1951)
9 GREEN, A A, *J biol Chem*, 158, 315 (1945)
10 GREEN, A A and CORI, G T, *J biol Chem*, 151, 21 (1943)
11 ILLINGWORTH, B and CORI, G T, *J biol Chem*, 199 653 (1952)
12 ILLINGWORTH, B, LARNER, J and CORI, G T *J biol Chem*, 199, 631 (1952)
13 LARNER, J ILLINGWORTH B, CORI, G T and CORI, C F, *J biol Chem*, 199, 641 (1952)

DISCUSSION

HOET Are you acquainted with the work of Madame Lamotte-Barillon in Paris? She finds that when the blood of patients with von Gierke's disease is injected into the toad or frog, large amounts of glycogen are deposited in the liver

GERTY CORI No I am not

LAZAROW Patients with glycogen storage disease frequently show a diabetic type of response to a glucose tolerance test How can you account for this abnormal tolerance to glucose in terms of a low glucose-6-phosphatase content of the liver? How can a low liver phosphatase level interfere with the removal of glucose from the blood stream?

GERTY CORI As the liver is already loaded with glycogen, it cannot deposit the normal amount of glycogen after a glucose meal The blood sugar level becomes very high and then falls to subnormal values during the fasting because the liver glycogen cannot be mobilized A case has come to autopsy at Columbia University in which the glucose-6-phosphatase is at the same low level as in my two cases

HOET Have you studied the glucose-6-phosphatase in embryonic or neonatal tissues?

GERTY CORI No The enzyme is present in kidney and liver but not in muscle or heart As the disease affects glycogen deposition in both groups of tissues, it would seem that this enzyme cannot be involved in cases of glycogen storage disease

LONG In some cases glycogen is deposited in the heart only and not in the liver

GERTY CORI I did not have occasion to study such a case

YOUNG How large a sample of glycogen do you need for studies of the kind you have made?

GERTY CORI 10–15 mg The glycogen is isolated in a high state of purity from 1–2 g of fresh tissue

WILHELMI Have you examined any of these abnormal glycogens by physical methods?

GERTY CORI They were studied in the ultracentrifuge by John Taylor The amylopectin-like material has a particle weight of about 500,000, which is smaller than that of normal glycogen The X-ray diffraction pictures have already been mentioned in my paper

LAZAROW Have you made any study of the branching in the macromolecular particulate glycogen which we isolated from the liver about ten years ago?

GERTY CORI I think one must not talk of this glycogen having a *molecular* weight but rather a *particle* weight It is very impure and contains many other substances

LAZAROW The sample of particulate glycogen which we prepared by physical methods contained 0·019 per cent phosphorus, 0 17 per cent nitrogen and 0 16 per cent of extractives soluble in alcohol-chloroform. It is obvious, therefore, that the [illegible]
lipid contam[illegible]
tion of glyc[illegible]

GERTY C[illegible]
which [illegible]

[illegible]
tainly d[illegible]
cells are [illegible]
against [illegible]
glycoge[illegible]
particul[illegible] [illegible] agents are used in the preparat[illegible] the particulate glycogen, there is little doubt that most of the glycogen that is stored in the liver cell exists in this macromolecular form

GERTY COR[illegible]

YOUNG H[illegible]
hormone bal[illegible]

GERTY CORI No, but we have not looked for one

LONG The only experimental situation comparable with your cases arises if large amounts of adrenal cortical steroids are given (as I discussed in my paper at the *Ciba Foundation Colloquium on Hormonal Factors in Carbohydrate Metabolism*, p 136, 1953)

GERTY CORI I would not expect the struct[illegible]
different in this case [illegible]

LON[illegible]
the hy[illegible] [illegible] to epinephrin[illegible]

GERTY CORI Russell and I could not confirm this observation

DE DUVE Your case 'MAS' is a most interesting one Did you measure the phosphatase activity in the kidney?

GERTY CORI No, I have not yet done so

DE DUVE This case also had a very low liver glycogen level

GERTY CORI The l[illegible]

level can rise in diabetes A normal kidney has a very small amount *about 0 2 per cent*

HOET In diabetic coma the kidney is full of glycogen and casts of pure glycogen can be seen in the tubules

GERTY CORI The comparison of histological estimations of the amount of glycogen with the amount determined chemically is usually satisfactory, but sometimes the histological determination gives a very erroneous idea of the amount present

LAZAROW Paul Ehrlich long ago showed that in diabetes the glycogen is deposited primarily in the proximal convoluted tubules Therefore the glycogen is unevenly distributed in the kidney and the overall concentration may be quite low

LONG Is glycogen deposited in the beta-cells of the pancreas at the same time as it is in the liver?

GERTY CORI We have not determined the glycogen content of the pancreas

CONN Glycogen is deposited in the liver in the early phases of alloxan diabetes Does alloxan inhibit glucose-6-phosphatase?

DE DUVE *Alloxan destroys the enzyme in vitro*

GERTY CORI It is a very sensitive enzyme Activity is retained for a considerable time so long as the structure of the tissue is preserved but it rapidly disappears as the tissue is ground up

GRIFFITHS Could the low levels of phosphatase activity have been due to the presence of an inhibitor?

GERTY CORI Inhibition of the enzyme is excluded because we mixed normal and abnormal preparations and observed no significant decrease in the activity of the normal We thought that an essential cofactor might have been absent but addition of boiled liver juice had no effect

GRIFFITHS What is the ratio of the enzyme concentration in the normal to that in the abnormal cases?

GERTY CORI In our two cases and the one at Columbia University (under the care of Dorothy Anderson) the enzyme was as good as absent In mild cases the concentrations ranged from one-third of normal to normal

CARL CORI The case at Columbia University had a hepatoma and the glycogen was higher in the hepatoma than in the rest of the liver Phosphatase was absent from both the hepatoma and the normal liver tissue

LOUBATIÈRES Professor Hoet has very aptly referred to the work of Madame Lamotte-Barillon As a result of experiments carried out in Kepinov's laboratory, she has put forward the hypothesis that the accumulation of glycogen in the liver is due to the inhibition of the glycogenolytic activity of adrenaline, this inhibition being ascribable to pituitary insufficiency

In 1944 I suggested a rather different explanation, namely, that von Gierke's disease is due to overactivity of the anterior lobe of the pituitary In fact I feel that this hyperfunction could at first result in von Gierke's syndrome and later give rise to diabetes mellitus This hypothesis was formulated following the work of Young and the research which I was carrying out at that time My experiments showed, in fact that a diabetogenic fresh total extract of the anterior lobe of the pituitary promotes the accumulation of considerable quantities of glycogen in the livers of normal dogs but not in those of totally depancreatized dogs Simultaneous injection of the extract and of insulin (in doses sufficient to overcome the diabetogenic action of the extract) results in the accumulation of glycogen in the livers of depancreatized dogs, however One may therefore conclude that, on the one hand, insulin is indispensable to the glycogen-forming action of the extract, and, on the other hand, von Gierke's disease is caused by hyperfunction of the anterior pituitary compensated by the secretion of a sufficient quantity of insulin by the pancreas.

Yet another argument in favour of our hypothesis is provided by the occasional cases in which von Gierke's disease progresses further In some cases, in fact this syndrome develops after puberty in the direction of diabetes mellitus Now it appears that at this age the diabetic action of an anterior pituitary extract is to some extent facilitated. In this connection, it would be interesting to determine whether hormonal changes of the kind which occur at puberty are capable of influencing the activity of the glucose-6-phosphatase which is modified in von Gierke's disease

GERTY CORI Those children which die as babies and those which survive and sometimes later develop a mild diabetes may not, in fact, be suffering from the same disease

HOET Was the growth of the 19-year old boy inhibited?

GERTY CORI Dr McQuarry informed me that this boy was slow in growing at first but grew rapidly between the ages of 14 and 15. He still has a flat blood sugar curve when given epinephrine and glucagon is also ineffective in provoking hyperglycaemia

DE DUVE I should like to comment on the use of the term '[illegible]' to describe the disease Glucose-6-phosphatase seems to be [illegible] to the microsomes and these particles are thought to be self-[illegible]

The deficiency may therefore be caused by a temporary failure of the particles to multiply There may not actually be a genetic difference.

LUKENS These studies indicate that physicians must recognize that enzymic disorders, as well as hormonal disorders, may be causes of disease Disease may also result from a disordered equilibrium amongst the enzymic components (as may be the case with hormones) rather than from a total absence of any one enzyme

GROWTH HORMONE AND GENERAL METABOLISM

By

A E WILHELMI

Department of Biochemistry, School of Medicine, Emory University, Emory, Georgia, U S A

INTRODUCTION

The availability of ample supplies of highly purified anterior pituitary growth hormone, as a result of improved methods for its isolation (47), has stimulated in recent years a large body of work with a wide variety of animals and tissues In a short paper with this general title it is impossible to deal comprehensively and critically with more than a fraction of the published work This account will therefore be concerned chiefly with recent work by Dr Russell and myself, in collaboration with Drs Fishman, Milman, Illingworth, Bondy and Welt This work has been concerned with the effects of growth hormone on a number of aspects of carbohydrate and fat metabolism and it is therefore fairly closely related to problems involving growth hormone and general metabolism Pertinent work by other investigators will be referred to, but not exhaustively, and it is hoped that any unseemly omissions will be brought to light in the discussion

Work with crude anterior pituitary extracts and partly purified fractions during the past twenty-five years has yielded a great variety of names for the actions of different pituitary preparations possessing varying but significant amounts of growth-promoting [illegible]

one of the known pituitary hormones, acting either independently or in concert with one or more of the other endocrine factors Dr Campbell has dealt elsewhere in this volume with the evidence relating the diabetogen[illegible] effect to gro[illegible] h[illegible] [illegible] Dr Fo[illegible]

[illegible]

trophic hormone (ACTH) to the glycotrophic effect

The deficiency may therefore be caused by a temporary failure of the particles to multiply There may not actually be a genetic difference

LUKENS These studies indicate that physicians must recognize that enzymic disorders, as well as hormonal disorders, may be causes of disease Disease may also result from a disordered equilibrium amongst the enzymic components (as may be the case with hormones) rather than from a total absence of any one enzyme

GROWTH HORMONE AND GENERAL METABOLISM

By

A E WILHELMI

Department of B ochemistry School of Medicine
Emory University Emory Georgia U S A

INTRODUCTION

The availability of ample supplies of highly purified anterior pituitary growth hormone as a result of improved methods for its isolation (47) has stimulated in recent years a large body of work with a wide variety of animals and tissues In a short paper with this general title it is impossible to deal comprehensively and critically with more than a fraction of the published work This account will therefore be concerned chiefly with recent work by Dr Russell and myself in collaboration with Drs Fishman Milman Illingworth Bondy and Welt This work has been concerned with the effects of growth hormone on a number of aspects of carbohydrate and fat metabolism and it is therefore fairly closely related to problems involving growth hormone and general metabolism Pertinent work by other investigators

fractions during the past twenty five years has yielded a great variety of names for the actions of different pituitary preparations

one of the known pituitary hormones acting either independently or in concert with one or more of the other endocrine factors Dr Campbell has dealt elsewhere in this volume with the evidence

trophic hormone (ACTH) to the glycotrophic effect

The deficiency may therefore be caused by a temporary failure of the particles to multiply There may not actually be a genetic difference

LUKENS These studies indicate that physicians must recognize that enzymic disorders, as well as hormonal disorders, may be causes of disease Disease may also result from a disordered equilibrium amongst the enzymic components (as may be the case with hormones) rather than from a total absence of any one enzyme

[illegible]

of insulin and of growth hormone on glycogen accumulation in the gastrocnemius, heart and diaphragm muscles of glucose-fed rats, observed that the rat diaphragm is more sensitive to insulin than either of the other muscles The effect of insulin alone was so large that no more glycogen accumulated in the diaphragm when insulin and growth hormone were given together The unusual sensitivity of the diaphragm to insulin may therefore make it difficult to observe direct anti-insulin effects on the uptake of glucose by this tissue

The evidence so far obtained indicates that the growth hormone is associated with those activities of the anterior pituitary that are concerned with the conservation of the supply of carbohydrate in the organism These actions appear to be expressed in two phases There is an early effect on the disposition of carbohydrate within the cell, characterized by a depression of the respiratory quotient, a diminution in the rate of carbohydrate oxidation and the preservation or enhanced accumulation of muscle glycogen Then a second phase develops more slowly, in which glucose uptake by the cells is diminished and the normal effect of insulin on the blood sugar is suppressed Whether these two phases of action represent stages in the development of a series of cellular processes regulated by growth hormone itself, or whether they represent quite different activities associated with the entity that we call purified growth hormone, cannot yet be decided The reorientation of the metabolic pattern of cells may not be a rapid process in all of its aspects and the earliest measurable changes may not be obviously related to those that occur later on In th[illegible]

[illegible]

may regain completely the normal capacity to synthesize fatty acids from [^{14}C]acetate (13), and that Park observed that the glucose uptake of isolated diaphragm tissue from hypophysectomized rats was most effectively reduced to normal after several daily injections of purified growth hormone (cited in 25) The problem of what are the phases of action of a hormone may there-

some hours In adrenalectomized rats or hypophysectomized dogs, growth hormone may induce a fatal hypoglycaemia In alloxan diabetic or partially depancreatized rats, on the other hand, the injection of growth hormone is followed by an immediate and sustained *rise* in the blood sugar level, an action which can be interpreted as a diabetogenic effect The lowering of the blood sugar level in normal animals by growth hormone *might be ascribed to a stimulation of insulin secretion by the* pancreas, in diabetic animals such a response does not occur and the action of the hormone in suppressing peripheral carbohydrate utilization predominates De Bodo, however, has observed that a *single injection of growth hormone into a newly depan-*creatized dog is followed by a fall in the blood sugar level, an effect which must depend upon the residual insulin in the animal rather than upon an increased secretion of insulin Another possibility is that growth hormone, by promoting protein synthesis, diminishes the rate of gluconeogenesis and of glucose output by the liver This effect would be small or absent in the diabetic animal, as insulin must be present for the nitrogen-retaining action of growth hormone to be exerted

Although the initial effects of growth hormone seem to involve a combined action with insulin, a state of resistance develops some hours after the injection of growth hormone, such that the hypoglycaemic action of a given dose of insulin is greatly reduced This effect has been observed in normal, diabetic and adrenalectomized diabetic rats (31) and in normal and hypophysectomized dogs (15, 26) This appears to be another aspect of the [illegible] action of the anterior pituitary [illegible]

[illegible] insulin action of growth hormone appears to be independent of the adrenal cortex A delayed effect of growth hormone on the uptake of glucose by *the isolated rat diaphragm has also been* observed by Krahl (25) Incubation with the hormone or injec-[illegible] effect on glucose [illegible] twenty-[illegible] about a [illegible] marked depression of glucose uptake The effect of insulin on this process was not diminished however, so that this action of

of differences in rates of peripheral utilization cannot be drawn from the data This point requires more careful experimental examination. It was also observed that growth hormone increased the ketonaemia of 16-hour fasted adrenalectomized rats (although the effect was much smaller than in normal animals), but that ACTH did not The ketogenic action of ACTH in [illegible] methods of assay, that growth hormone is entirely absent from ACTH protein preparations and the effects observed may have been due to the presence of a small amount of growth hormone, augmented by the adrenal stimulation It may also be true, however, that the induced adrenal activity may have been far greater and more continuously effective than the doses used in previous unsuccessful experiments

The observations of these authors have been repeated and extended by Bondy and Wilhelmi (6) It was shown that the injection of 5 mg. of purified growth hormone into 40-hour fasted normal rats brings about in two to three hours a marked increase in the concentration of the blood ketones (from 49 to [illegible] mg of ACTH induces a [illegible] (from 35 to 44 mg. per [illegible] duction in isolated liver slices taken from these animals was not greater than that in liver slices from untreated fasted controls Ketone body production by liver slices from hypophysectomized animals was much less than normal and identical with that of liver slices from thyroidectomized rats The rates could be restored to normal by treatment of the hypophysectomized animals with thyroxine, but the [illegible] It was noted that the ketone body production by liver slices from 40-hour fasted adrenalectomized rats was significantly lower than that of liver slices from 18-hour fasted rats and that the ketonaemia of adrenalectomized rats did not increase significantly from the eighteenth to the fortieth hour of a fast

These observations have been confirmed in part and extended

fore not be solvable by attempting to break it down into its components, it may require a much better understanding of the nature of intracellular organization

EFFECTS ON KETOGENESIS

Since growth hormone promotes nitrogen retention and assists conservation of the carbohydrate stores, it may be expected that its action is also associated with an increased utilization of fat in order to provide the energy which is no longer available from the other major foodstuffs Evidence for this has been provided by several authors who have shown that in animals treated with crude anterior pituitary extract or with purified growth hormone the proportions of protein, water and salts in the body increase, and the proportion of fat is much diminished (27, 28, 30, 48)

More direct evidence for an effect of the anterior pituitary on fat metabolism was provided by Burn and Ling, who showed that the injection of crude anterior pituitary extract into fasting rats greatly increased their fasting ketonuria (11) This effect has since been studied by a number of workers, some of whom have attempted to separate the ketogenic activity from other actions of anterior pituitary extract (12, 40) The most active fractions have usually contained significant amounts of growth-promoting activity The ketogenic effect is also associated with an increased mobilization of fat to the liver (12)

An association of ketogenic action with highly purified pituitary hormone was demonstrated by Bennett *et al* who showed that purified growth hormone and protein ACTH, injected into fasting normal rats, increased the ketonuria in the last 48 hours of a 72-hour fast, and increased the ketonaemia three hours after injection into 24-hour fasted rats (3) Lactogenic hormone was without effect in these circumstances It was concluded that the effect of growth hormone and of ACTH was on the rate of ketone body production by the liver, since the rate of fall of the blood ketone level of the treated animals, in thirty minutes after evisceration and nephrectomy, was not different from that seen in control animals The initial levels were only moderately high, however (30–45 mg per cent), the rate of fall was rapid (15–30 mg per cent per hour) and the individual values were variable, so that a definite conclusion about the significance

almost completely suppressed in the diabetic rat and that in the rabbit insulin markedly accelerates the conversion of carbo hydrate to fat in the liver These effects have since been confirmed by other workers

[illegible]

maintained in a constant concentration in the body water was studied in plateaued female adrenalectomized rats normal rats and in normal rats treated with purified growth hormone and [illegible]

animals treated with growth hormone and with ACTH in which it diminished Groups of three animals in each series were sacrificed on the first second fourth and eighth days of exposure to deuterium In the adrenalectomized animals the rates of incorporation of deuterium into both the liver and the carcass

absence of the adrenal cortex therefore a larger proportion of the dietary carbohydrate is utilized by conversion to fat which is subsequently oxidized Growth hormone and ACTH both have the effect of inhibiting the conversion of fat to carbohydrate Further work is required in order to determine which of the hormones has the primary action

From other instances some of which have been cited above of the permissive action of the adrenal cortex in the realization of the effects of growth hormone the opinion might be ventured that growth hormone is the primary factor This is supported by the result given by the adrenalectomized animal whose own pituitary did not prevent the acceleration of fat synthesis The relation of this effect of growth hormone to the diabetogenic R Q -depressing and carbohydrate-conserving actions is apparent The effect also leads to the suggestion that in the diabetic animal the absence of insulin may not be the only factor involved in the suppression of lipogenesis from carbohydrate This has been nicely demonstrated by Brady Lukens and Gurin who have

by Tepperman and Tepperman (43) Ketone body production, *which is only slightly diminished in liver slices from rats less than fifteen days after hypophysectomy, is restored to normal by injection of purified growth hormone three hours beforehand In rats hypophysectomized for more than thirty days, ketone body production was much less than normal and it was not affected by previous injection of growth hormone If, however, both growth hormone and a small dose of cortisone (which by itself is ineffective) were injected together, the normal rate of ketone body production by the liver slices was restored When eight slices of liver tissue from *fed* normal rats were rapidly prepared and alternate slices incubated in a medium containing growth hormone, ketone body production was in each instance greater in the hormone-treated slice Boiled hormone or crystalline bovine serum albumin were ineffective The *in vitro* effect was consistently obtained with old rats weighing 300–400 g and with two different growth hormone preparations With liver tissue from young 200 g rats the effect could not be obtained Two other growth hormone preparations which I supplied have been ineffective *in vitro* in repeated trials The factors responsible for these differences have not yet been determined

On the basis of the present evidence, the role of the growth hormone in ketone body production is not certainly established The indications that the age of the animal and the levels of activity of the thyroid and the adrenal cortex may influence ketone body production, and the technical difficulties in devising a stable and responsible *in vitro* system, make it necessary to reserve judgment about the identity of the ketogenic principle with growth hormone

EFFECTS ON THE CONVERSION OF CARBOHYDRATE TO FAT

The major pathways for the disposal of carbohydrate in the body are by storage as glycogen oxidation and transformation to fat Boxer and Stetten, in the course of studying glycogen turnover in rats on a high carbohydrate diet with deuterium in the body water, have shown that a significant fraction (about one-third) of the daily carbohydrate intake may be disposed of by conversion to fat and subsequent oxidation (7, 42) They have also shown that the synthesis of fat from carbohydrate is

A number of investigators have recently studied the last-named process in detail The first suggestion that some aspect of carbohydrate utilization may be related to the synthesis of fatty acids from acetate lies in the observation of Bloch that, in rat liver slices, the labelled carbon of acetate, present alone as substrate, is found mainly in newly synthesized cholesterol, but that if pyruvate is also present, the larger portion of the labelled acetate carbon is found in newly synthesized fatty acids (5) More recently, Brady and Gurin, and Chaikoff and his co-workers have observed that in liver slices from diabetic rats the conversion of both glucose and acetate to fatty acids is greatly inhibited, that this process is restored to normal by treatment with insulin and facilitated in liver tissue from normal rats by treatment with insulin (1, 8, 9, 13, 14, 17, 18, 32) The proportion of [^{14}C]acetate oxidized to carbon dioxide is not very different in any of these instances Brady and Gurin have put forward the hypothesis that the pathway of breakdown of fatty acids to acetate is different from the pathway followed by acetate in the formation of fatty acids (9) There is therefore a cycle of acetate production and removal and it is the return half of this cycle which is blocked in the absence of insulin and which, as Brady, Lukens and Gurin have shown, is reopened in the Houssay cat (10) This means that it may also be inhibited by the anterior pituitary Another point of the argument is provided by the observation of Baker *et al* that, in liver slices from diabetic rats previously fed a diet rich in fructose, the rate of incorporation of [^{14}C]acetate into fatty acids in the presence of fructose is substantially normal and appears to be independent of insulin (1) Fructose therefore appears to follow a metabolic pathway connected with the incorporation of acetate into fatty acids, this pathway is only open to glucose in the presence of insulin (and perhaps in the absence of the anterior pituitary as well) In this connection, one may also recall the observation that in liver slices from normal rats fructose increases the oxygen uptake, the respiratory quotient and the degree of saturation of the liver fatty acids, but glucose does not (16) In rats trained to eat all of their daily food requirement in two hours, which appears to be possible only when there is a great increase in the capacity for synthesizing fat from carbohydrate, the response of the liver *slices to glucose as a substrate*

shown that the rate of incorporation of [^{14}C]acetate into fatty acids, which is nearly zero in liver slices from diabetic cats is restored towards the normal in liver slices from otherwise untreated hypophysectomized diabetic (Houssay) cats The amelioration of diabetes in the Houssay animal may therefore be attributed in part to the relief of an inhibition of an important metabolic pathway of carbohydrate utilization

Although there are still many points of uncertainty, the main

on the several aspects of carbohydrate and fat metabolism that have been discussed in the foregoing pages There is also a good body of evidence, based on the work of Weil and Ross and of Levin (29, 44), that growth hormone may be a primary factor in the fat-mobilizing action of the anterior pituitary If this be accepted for purposes of argument, what general hypothesis can be proposed to relate all of these actions of the hormone? A suggestion arises out of the modern concept of the continuous metabolism of the foodstuffs and from current experimental work on the intermediary metabolism of fat and carbohydrate

A GENERAL PLAN OF THE MODE OF ACTION OF GROWTH HORMONE

The breakdown of fatty acids in the liver has been shown to take place by a process of β-oxidation leading to the production of two carbon units similar to acetic acid (but probably present in an 'active' form, such as acetyl phosphate or acetyl coenzyme A The oxidative breakdown of carbohydrate leads, over the now familiar pathway of the glycolytic cycle, to pyruvate, which may be oxidatively decarboxylated to form 'acetate'. In the liver the acetate units may

(i) be coupled with one another at random to form acetoacetate, which may undergo partial reduction to β-hydroxybutyrate,

(ii) be coupled with oxaloacetate and oxidized via the steps of the tricarboxylic acid cycle,

(iii) be used in the acetylation of foreign amines,

(iv) be used in the synthesis of cholesterol, or

(v) be utilized in the synthesis of fatty acids

(ii) In the peripheral tissues, the absence of insulin prevents the glucose which enters the cell from forming the essential intermediate and limits its functions to an osmotic one in the fluids bathing the cell There may therefore be an increase in the net utilization of fat This may be accentuated by the injection of growth hormone, since the tissue glycogen may be diverted in

synthesis, brings about

(a) a greater accumulation of muscle glycogen, and

(b) a depression of the respiratory quotient (owing to the suppression of a process with a high respiratory quotient)

R Q -depressing effect in animals fed glucose, and that when the two hormones are given together in these circumstances the gastrocnemius muscle glycogen attains levels rarely seen—in excess of one per cent (35)

(iii) The mobilization of fat from the depots to the liver, which is also associated with the action of growth hormone, requires the assumption that the availability of carbohydrate (which is increased by insulin and limited by growth hormone) for the

ing fat synthesis in these cells with the prior accumulation of glycogen is indeed suggestive that the ebb and flow of fat in adipose tissue as the animal cycles between the fasting and fed states may also be related to a carbohydrate-coupled 'feedback' in fatty acid metabolism

Finally, one may ask what relationship, if any, can exist between the nitrogen-retaining effect of growth hormone and its action in limiting the utilization of an intermediate of carbohydrate metabolism for fat synthesis? It is most tempting to

is identical with the response to fructose seen in the untrained rat

If the hypothesis of Brady and Gurin is elaborated by addition of a few simple and reasonable postulates, a fairly well integrated plan of the action of growth hormone in metabolism can be constructed These postulates are

(i) From the concept of the continuous metabolism of the foodstuffs, and the body of experimental data in support of it it may be assumed that in all of the tissues of the normal animal there is continuous breakdown and resynthesis of fatty acids and a continuous and rapid conversion of carbohydrate to fatty acids

(ii) The amount of fatty acid undergoing complete oxidation or conversion to ketone bodies is the *net* result of

(a) the oxidation of fatty acids to two-carbon units and of

(b) the reassimilation of two-carbon units into fatty acids, *a process which is coupled in an obligatory fashion with the utilization of carbohydrate in the synthesis of fatty acids*

(iii) Since fructose may promote the assimilation of [^{14}C]acetate into fatty acids in diabetic animals, while insulin is required

produced from fructose, which plays a direct part in the conversion of carbohydrate to fatty acids and the associated assimilation of acetate into fatty acids

(iv) Growth hormone inhibits the utilization of this intermediate for fatty acid synthesis and this action may be augmented by cortisone

The consequences of this hypothesis are as follows

(i) Depletion of the carbohydrate stores as in the liver of the

the liver produces glucose from protein, and this glucose may be diverted in part to the 'feedback' The administration of growth hormone can suppress this utilization It therefore not only increases the availability of glucose to the peripheral tissues, but also results in a net increase in ketone body production—the ketogenic effect

13 CHERNICK, S S and CHAIKOFF, I L., *J biol Chem*, 188, 389 (1951)
14 CHERNICK, S S, CHAIKOFF, I L and ABRAHAM, S, *J biol Chem*, 193, 793 (1951)
15 DE BODO, R C, KURTZ, M, ANCOWITZ, A and KIANG, S P, *Amer J Physiol*, 163, 310 (1950)
16 DICKERSON, V C, TEPPERMAN, J and LONG, C N H, *Yale J Biol Med*, 15, 875 (1943)
17 FELTS, J M, CHAIKOFF I L and OSBORN, M J, *J biol Chem*, 191, 683 (1951)
18 FELTS, J M, CHAIKOFF, I L and OSBORN, M J, *J biol Chem*, 193, 557 (1951)
19 GREAVES, J D, FREIBERG, I K and JOHNS, H E, *J biol Chem*, 133, 243 (1940)
20 GROLLMAN, A, *Amer J Physiol*, 122, 460 (1938)
21 HERRING, V V and EVANS, H M, *Amer J Physiol*, 140, 452 (1943)
22 ILLINGWORTH, B A and RUSSELL, J A, *Endocrinology*, 48, 423 (1951)
23 ILLINGWORTH, B A and RUSSELL, J A unpublished experiments
24 INGLE, D J, *Recent Progr Hormone Res*, 6, 159 (1951)
25 KRAHL M E, *Ann N Y Acad Sci*, 54, 649 (1951)
26 KURTZ, M, DE BODO, R C, KIANG, S P and ANCOWITZ, A, *Proc Soc exp Biol, N Y*, 76, 21 (1951)
27 LEE, M O and AYRES, G B, *Endocrinology*, 20, 489 (1936)
28 LEE, M O and SCHAFFER, N K, *J Nutr*, 7, 337 (1934)
29 LEVIN, L and FARBER R K, *Recent Progr Hormone Res*, 7, 399 (1952)
30 *LI*, C H, *SIMPSON*, M E and *EVANS* H M, *Endocrinology*, 44, 71 (1949)
31 MILMAN A E and RUSSELL, J A *Endocrinology*, 47, 114 (1950)
32 OSBORN, M J, CHAIKOFF, I L and FELTS, J M, *J biol Chem*, 193, 549 (1951)
33 RECANT, L, *Fed Proc*, 11, 272 (1952)
34 RUSSELL, J A, *Amer J Physiol*, 121, 755 (1938)
35 RUSSELL, J A, *Amer J Physiol*, 124, 774 (1938)
36 RUSSELL, J A, *Endocrinology*, 22, 80 (1938)
37 RUSSELL, J A, *Amer J Physiol*, 128, 552 (1940)
38 RUSSELL, J A, unpublished experiments
39 RUSSELL, J A and WILHELMI, A E, *Endocrinology*, 47, 26 (1950)
40 SHIPLEY, R. A, *Amer J Physiol*, 141, 662 (1944)
41 SHIPLEY, R A and FRY, E G, *Amer J Physiol*, 135, 460 (1942)

synthesis It does, however, seem worthy of consideration for the reasons that the retention of nitrogen is a matter of *collaboration* between growth hormone and insulin and that it cannot simply be a matter of making cellular energy available

[illegible] for suggest- is due in the l Gunn, has, I think, both of these virtues The poet Wallace Stevens, in a little poem called *Thirteen Ways of Looking at a Blackbird*, said, in the sixth way

'When the blackbird flew out of sight
It marked the edge
Of one of many circles'

The subject of our discussion has, I fear, a tatterdemalion aspect most foreign to that of the trim and dapper blackbird It must be acknowledged as well that this attempt to lend it a neater and more harmonious habit can only 'mark the edge of one of many circles'

REFERENCES

1 BAKER, N, CHAIKOFF, I L and SCHUSDEK, A, *J biol Chem* 194, 435 (1952)
2 BENNETT, L L, *Endocrinology*, 22, 193 (1938)
3 BENNETT, L L, KREISS, R E, LI, C H and EVANS, H M, *Amer J Physiol* 152, 210 (1948)
[illegible] 6, 24 (1945)
[illegible] 6, 245 (1950)
7 [illegible] J biol Chem 155 237 (1944)
8 [illegible] 1950)
9 [illegible] 1950)
10 [illegible] *ol Chem*, 193, 459 (1951)
11 BURN, J H and LING, H W, *J Physiol*, 69, xix (1930)
12 CAMPBELL, J and DAVIDSON, I W F, *J biol Chem*, 189, 35 (1951)

did you determine the amounts of fat and acetate present at the same time as you estimated the sugar and glycogen?

WILHELMI A direct estimation of either fat or acetate was not carried out Hastings, using ^{14}C-labelled precursors, studied the distribution of radioactivity in the protein amino-acids and the fatty acids and found that not all of the radioactivity was incorporated into the glycogen, some went into the protein and fat

CARL CORI I believe that only two or three per cent was incorporated into protein and fat

LUKENS In Krahl's unpublished experiments the fat metabolism of muscle was not studied, he worked only with the liver Other tissues should be studied

WILHELMI Wertheimer reports that the uptake of lipids from serum by the diaphragm occurs only if the glucose content of the serum is nil Uptake of lipids also occurs if sera from diabetics are used which have a high lipid content Addition of glucose (50 mg per cent) and also of acetoacetic acid, inhibits the uptake This is rather an interesting new approach.

YOUNG Is there any effect of growth hormone on the availability of glycerol for fat synthesis?

WILHELMI This is not known and must be tested experimentally In the ruminant, Folley has shown that glycerol is the limiting factor in the synthesis of fatty acids from acetate and that insulin is without effect in the presence of these substrates

YOUNG Stewart in our laboratory, finds that glycerol has a strong antiketogenic effect

WILHELMI Is glycerol as efficient as other precursors of glycogen, Professor Cori?

CARL CORI Glycerol is a good glycogen precursor in the liver Folley reported at the *Ciba Foundation Colloquium on Hormonal Factors in Carbohydrate Metabolism* that insulin probably has no effect on the conversion of two-carbon units to fatty acids

of glucose into the tissue

Incidentally, does insulin modify the glycostatic effect of growth hormone?

WILHELMI No Russell found that large doses of insulin *plus* anterior pituitary extract had little effect on the respiratory quotient but that there was a large effect on the glycogen content of the muscle, that is, growth hormone did not antagonize the effect of insulin on the uptake of glucose

42 STETTEN, D, Jr and BOYER, G E, *J biol Chem*, 155, 231 (1944), 156, 27 (1944)
43 TEPPERMAN, J and TEPPERMAN, H M, *Ann N Y Acad Sci*, 54, 707 (1951)
44 WEIL, R and ROSS, S, *Endocrinology*, 45, 207 (1949)
45 WELT, I D and WILHELMI, A E, *Yale J biol Med*, 23, 99 (1950)
46 WERTHEIMER, E and SHAPIRO, B, *Physiol Rev*, 28, 451 (1948)
47 WILHELMI, A E, FISHMAN, J B and RUSSELL, J A, *J biol Chem*, 176, 735 (1948)
48 YOUNG, F G, *Biochem J*, 39, 515 (1945)

DISCUSSION

LONG Does the increased availability of two-carbon units resulting from the administration of growth hormone lead to an increase in the amount of cholesterol synthesized?

LUKENS Brady and Gurin have shown that in the diabetic organism, in which fat synthesis is blocked, the synthesis of cholesterol proceeds at the *normal* rate In fact, cholesterol synthesis does not seem to be affected by any hormonal manipulation

LONG Dr Conn has shown that a change in the concentration of cholesterol in the blood is produced by administration of ACTH

LAWRENCE If we accept that growth hormone stimulates fat catabolism, there is no need to look for a primary effect on the production of ketones And if fat is being anabolized at the same time, resistance to insulin will develop because its action in stimulating the formation of fat is being prevented

WILHELMI A high rate of fat catabolism and a high level of ketosis are coupled with a limited rate of carbohydrate utilization The rate

Because the liver also produces sugar from non-sugar precursors, the flow of carbon from these sources may be towards carbohydrate and less may be oxidized by the citric acid cycle In addition, if growth hormone has a strong action in the peripheral tissues, it will not be antagonized by insulin and will depress the rate of synthesis of fatty acids Oxidation will then be the only pathway remaining for the disposal of acetoacetic acid One must determine experimentally whether growth hormone has an effect on the rate of peripheral utilization of ketones—an action which is predicted by the scheme I have put forward

LAWRENCE In your experiments with diaphragms, Dr Wilhelmi,

RELATIONSHIPS OF GROWTH HORMONE TO DIABETES

By

JAMES CAMPBELL, J S MUNROE, H R HAUSLER and I W F. DAVIDSON

Department of Physiology, University of Toronto, Toronto, Canada

INTRODUCTION

Certain influences of the growth hormone of the anterior pituitary gland on metabolism will be discussed herein The studies from which the findings are derived can be grouped under three main headings, which are —The production of [illegible]

[illegible] deprivation in diabetic animals Metabolic interrelationships revealed by these studies will be discussed, in particular the relationship of growth hormone to the insulin secretory activity of the pancreas

The growth hormone was isolated from bovine anterior pituitary glands by Li, Evans and Simpson (30) The product did not contain detectable amounts of thyroid-stimulating, adrenocorticotrophic (ACTH), prolactin, luteinizing or follicle-stimulating activities, and by solubility tests and electrophoretic analyses appeared to be homogeneous This achievement crowned the endeavours towards this end which had been main- [illegible] Wilhelmi, [illegible] pre- [illegible] (47), [illegible] n and his associates for the separation of protein fractions from plasma The hormone obtained by these methods from bovine glands is a protein. Li and Evans have found at least fifteen amino-acids in hydrolysates and have accounted thereby for 93 per cent of the protein by weight (29) The molecular weight, as determined by various methods, is given as 39,000 to 49,000 (29, 46) By ultracentrifugation, Li and Moskowitz (1949) found the molecular weight to be 44,000 and determinations on the preparation of Campbell and Davidson agree with this value. The isoelectric

CARL CORI This separates the glycostatic action of growth hormone from its effect on glucose uptake Recant (unpublished) tested several preparations of growth hormone which had retained their depressant action on the respiratory quotient but which had lost their growth-promoting activity (The effect on the respiratory quotient is apparently not due to growth hormone itself, therefore) It was found that there is a substance of pituitary origin in the blood of a diabetic rat which consistently inhibits the uptake of glucose by the isolated diaphragm (as I mentioned in the discussion following Dr Long's paper) This effect on the entry of glucose into the tissues is different from the effect on the respiratory quotient, the former is antagonized by insulin, whereas the latter is not

WILHELMI We cannot yet explain satisfactorily why the injection of growth hormone diminished the effect of a subsequent dose of insulin Did Recant test for the depressant action on the respiratory quotient in the intact animal?

CARL CORI Recant used three types of preparation intact animals the diaphragm removed after treatment with growth hormone, and the isolated diaphragm treated *in vitro* with growth hormone In all of these the increase in respiratory quotient occurring in the presence of glucose was suppressed

DE JONGH There is a great contrast between the effects produced in a hypophysectomized animal according to whether glucose is given orally or by injection (see Professor Gaarenstroom's paper)

WILHELMI This is probably related to the rate of absorption of the glucose You would expect it to be slower in hypophysectomized animals *In our animals plenty of time was allowed for absorption.*

DE JONGH There was probably no effect due to differing rates of absorption in our experiments

[illegible] of
[illegible] ids
[illegible] lly
[illegible] in
[illegible]

HOET Do any temperature changes occur in hypophysectomized animals when large amounts of carbohydrate are given?

RUSSELL No fall in temperature was seen in our experiments It was not checked very carefully however, there might have been a small change Starch was used so that the carbohydrate would be absorbed at a slow rate

prepared by the first of these methods (DP) had growth-promoting activity which exceeded that of the preparation obtained by the procedure of Wilhelmi *et al* (47) The product was however, not pure The ACTH activity was of the order of 1 per cent of the International Standard (La-1-a) The thyroid stimulating activity was equivalent to 0 10 U S P units per mg, that is, it contained thyrotrophin activity equivalent to 10 per cent of that of the U S P temporary reference standard Lactogenic hormone activity equivalent to about 5 per cent of the International Standard was present

The material prepared by the third method (DKP) had the highest growth-promoting activity and the highest degree of purity No thyroid stimulating activity could be detected in these preparations by determination of the uptake of radio-iodine in hypophysectomized rats (10) No thyroid stimulating and no gonadotrophic activity was found by histological methods when the preparation was administered to hypophysectomized rats in large doses A possible but very slight adrenocorticotrophic effect was noted

The WP preparation was analysed electrophoretically at pH 9 9 and 4 0 by Dr A E Wilhelmi, whose results indicated that it was 90 per cent pure, or better, and established the identity of the main component with that of his purified growth hormone [illegible]

[illegible]

These preparations have been analysed in the Spinco ultracentrifuge by Dr A F Graham and by Mr K A B Degen of the Connaught Medical Research Laboratories The sedimentation [illegible] deter- [illegible] The [illegible] ation procedure is estimated by the ultracentrifugal analyses to be greater than 99 per cent

DIABETOGENIC EFFECTS OF GROWTH HORMONE

It was found by Cotes Reid and Young that highly purified growth hormone was diabetogenic in cats (16) Houssay and

point is pH 6 8 The purified material is soluble in dilute salt solution above pH 4, but is most soluble at an alkaline reaction The growth-promoting activity is said to be least labile at about pH 8 Raben and Westermeyer have, however, obtained [illegible]

have been obtained from bovine anterior pituitary glands by three methods In the first, growth hormone was prepared in crystalline form by the method of Wilhelmi *et al* (47) A further purification step was introduced into this method by us, the product being termed 'WP' (9) When tested for growth-promoting activity in hypophysectomized young rats by the body weight and by the tibia tests, this product gave responses which were about the same as those obtained by Li and his associates with isolated growth hormone (21, 30) It should be realized, however, that a number of variables enter into these tests, such as strain of rat, environmental temperature, diet etc., so that the results obtained in different laboratories may not be directly comparable The preparation contained minor but demonstrable amounts of other active principles Adrenocorticotrophic activity was present to the extent of 2 per cent [illegible]

on the signs of diabetes in dogs (9) We are much indebted to Professor H M Evans and Dr C W Asling for biological tests which showed detectable amounts of thyroid-stimulating hormone ($\pm$), *and of interstitial cell-stimulating hormone* ($\pm$ *to* $+$) were present, but that follicle-stimulating hormone could not be demonstrated In these tests, young hypophysectomized female rats were given a total of 2 mg of the preparation in four days, 24 hours after the last injection the animals were sacrificed, the tissues were weighed and examined histologically Prolactin activity was not found in systemic tests on squabs by Lyons' method (33) Similar results were obtained in this laboratory

The other two methods of preparation, to be reported by Campbell and Davidson in a future publication, were similar in design to the previous methods The highly purified material

tion and vacuolation with evidence of atrophy (7 18 32 42 43) Insulin could be extracted from the pancreases of meta-hypophyseal diabetic dogs but in amounts which were very small compared with the normal (5 7 35) It was reported by Ham and Haist that in animals given diabetogenic extracts the fall in the amount of insulin extractable from the pancreas paralleled a progressive degranulation of the beta-cells (24) In dogs with temporary pituitary diabetes the extractable insulin content of the pancreas was very low after eight days of injection but the normal content was rapidly regained on ceasing the injections whereas in permanent diabetes the insulin content remained low From results obtained by grafting the pancreas

Houssay and Anderson produced permanent diabetes by administering growth hormone preparations to the partially depancreatized dog (26) It has been demonstrated in our laboratory that permanent diabetes can be produced in intact dogs by very highly purified growth hormone Permanent diabetes has been produced in six dogs Two of these (P and W) had previously been given growth hormone which produced temporary diabetes and prior to this had been used to test the diabetogenic activities of other pituitary preparations After an interval of fifteen weeks during which the blood sugar level was normal and the urine was free of sugar growth hormone prepared according to the method of Wilhelmi *et al* (47) was administered Twenty-seven days of injection were required to render dog P

temporary diabetes elicited thereby had been absent for several

Anderson found that purified growth hormone had more pronounced diabetogenic effects in partially depancreatized dogs cats, rats and batrachians than had prolactin or ACTH (26) In hypophysectomized dogs, De Bodo *et al* found that the administration of growth hormone increased resistance to insulin and impaired tolerance to intravenously injected glucose (17) *Campbell et al found that the administration of highly purified* growth hormone produced temporary diabetes in dogs (9) Hyperglycaemia glycosuria polyuria and polydipsia occurred within two to four days of injection of 3 5 mg per kg body weight per day Ketonuria and lipaemia usually appeared later in these animals It has also been shown that in intact dogs given growth hormone the extractable insulin content of the pancreas is reduced and that marked degranulation of the beta-cells of the islets of Langerhans occurs (11)

Certain of the characteristics of temporary pituitary (idiohypophyseal) diabetes that can be produced by the administration of whole or partially purified anterior pituitary extracts have been described by Houssay and Biasotti (27), Young (49) and others The diabetes elicited by purified growth hormone in dogs is so far indistinguishable from this idiohypophyseal diabetes in respect of the nature and intensity of the responses elicited Campbell *et al* concluded that the major part of the diabetogenic activity of bovine anterior pituitary glands can be attributed to the growth hormone that they contain (9) The results indicated [illegible] the growth

to this hormone as *the* diabetogenic factor, since ACTH has definite, though relatively small, hyperglycaemic and glycosuric effects in dogs It is probable that ACTH and possibly other active principles of the pituitary gland also, will influence the responses of animals to mixtures of these principles

PERMANENT DIABETES PRODUCED BY GROWTH HORMONE

It was discovered by Young that when fresh saline extracts of the anterior pituitary gland were given to dogs for a sufficiently

polyuria and caused a rapid gain in body weight The improve- [illegible] well-being of the [illegible] ppeared that in the [illegible] given in order to prevent collapse due to emaciation and steadily advancing acidosis When the diabetes was controlled by insulin, so that [illegible]

ence is significant

Histological examination of the pancreases from these dogs by Dr W S Hartroft and Mr W Wilson showed extensive atrophy of islet tissue, with particularly severe degeneration of the beta-cells The insulin extractable from the pancreases was assayed by Dr G A Wrenshall and was found to be very low, 0 5 per cent of the normal amount in dogs P and W

These results show that highly purified growth hormone produced permanent diabetes in dogs and that the thyroid-stimulating, follicle-stimulating and luteinizing hormones and prolactin were excluded as necessary participants in the result The trace contamination by ACTH was probably negligible in effect in these preparations This permanent diabetes produced by growth hormone is the same in all the important features studied, as the metahypophyseal diabetes previously produced by crude or partially purified preparations The results provide a particularly striking demonstration of the diabetogenic effect of growth hormone

It has been reported by Condliffe and Li that the growth-promoting activity may not be affected by partial hydrolysis of growth hormone with carboxypeptidase (15) Similar results have been obtained by Reid, who showed also that the altered protein may possess diabetogenic activity (41)

THE PANCREATIC LESION OF PERMANENT PITUITARY DIABETES AND ITS AETIOLOGY

As was mentioned above, the histological changes in the pancreases of dogs made permanently diabetic with growth hormone

weeks before the Wilhelmi hormone preparation was administered After twenty-six and thirty-six days of injection respectively, these animals became permanently diabetic (13) One of these dogs (B) died, from unknown cause, four days after the

insulin was extractable from the pancreas, and the beta-cells of the islets of Langerhans were scanty and poorly granulated

The last two dogs (T and M) were not treated in any way before administration of the DKP and the DP preparations of growth hormone respectively Munroe and Chaikoff in our laboratory produced permanent diabetes in these dogs after thirty-seven and fifteen days of injection respectively (39) Some of the data relating to these animals are shown in Table I

TABLE I

createctomy (from ref. 14

Dog	Induction period		Metahypophyseal diabetes				
	Growth hormone preparation		Without insulin	Insulin requirement (units per day)			
	Dosage (*mg /kg body wt /day*)	Duration of *injections* (days)	(days)	Before pancreatectomy		After pancreatectomy	
				IZ*	PZI*	IZ*	PZI*
P	3 03	27	44	6	4	12	6
W	3 5	3	45	16	8	16	8
B	3 5	26	4				
J	3 5	36	52	8	4		

*IZ Insulin-zinc PZI Protamine zinc insulin.

The diabetes endured in these animals, *after cessation* of the injections, without signs of diminution of intensity No decreases in severity of the hyperglycaemia, polyuria or glycosuria were found during periods of over forty days When insulin was administered thereafter, the insulin requirements *did not* change appreciably—for a year in the case of dog J

The administration of insulin to the permanently diabetic dogs reduced the hyperglycaemia, glycosuria, ketonuria and

depancreatized dogs were given large meals, the islets in the remaining pancreatic tissue became degranulated, hydropic and finally atrophic (1) Diabetes appeared in these dogs If the diet of the partially depancreatized dogs was restricted, however, these changes in the beta-cells of the islets did not occur, and diabetes did not develop Thus, overstraining the capacity of the remaining islets caused degeneration of the beta-cells, and finally the changes became irreversible These degenerative

[illegible]

cause of metahypophyseal diabetes that, at some period during the earlier stages of induction of diabetes by pituitary extracts and as now transpires, by growth hormone there may be a stimulation of the beta-cells and an increase in the rate of secretion of insulin from them As will be shown below, evidence in support of this may be deduced from further experimental results It must be admitted at the outset, however, that we have not yet obtained direct evidence in favour of the hypothesis

RELATION OF THE GROWTH HORMONE TO THE PANCREATIC ISLETS

Milman and Russell found that injections of a growth hormone preparation in fasting rats produced prolonged hypoglycaemia (38) In adrenalectomized animals the injections produced hypoglycaemia, with convulsions and death In alloxan diabetic rats the blood sugar level rose following the injections In the opinion of these authors the simplest explanation for the results was that the growth hormone had stimulated secretion of insulin from the islet tissue Further experiments showed that both insulin and growth hormone decreased the concentration of amino-acids in the blood Since growth hormone was effective in reducing the concentration of amino-acids in the blood of diabetic rats, it appeared that insulin liberation could not explain this effect of growth hormone

coincide with those that have been described in metahypophyseal diabetic dogs by Richardson and Young (43), Campbell and Best (7), Ham and Haist (24) and others The acinar tissue appears normal, but the islets are small in size and few in number In the remaining islets there are few beta-cells in proportion to the number of alpha-cells The beta-cells are degranulated and have the appearance of being atrophic The lesion of the pancreas produced by the growth hormone reduces the amount of islet tissue and the numbers of both the alpha- and beta-cells The *beta-cells, by histological observations, are most severely injured* and the very small amount of insulin that can be extracted from the pancreas of the metahypophyseal diabetic dog supports these observations The *essential defect in metahypophyseal diabetes* is apparently the atrophy of the beta-cells with consequent failure of the islets of Langerhans to secrete insulin at an adequate rate The efficacy of insulin administration in restoring apparently normal metabolic conditions in metahypophyseal diabetes supports this interpretation

The aetiology of the islet lesion caused by growth hormone is a matter of much interest Anderson and Long found that the addition of growth hormone to the fluid perfusing the pancreas of the rat reduced the amount of insulin secreted by the organ in response to glucose, and concluded that the growth hormone had a direct inhibitory effect on the beta-cells (2) It should be recognized, however that the organ was removed from the influences of the other tissues of the body in these experiments Houssay *et al* also appear to incline to the view that the pituitary diabetogenic factor inhibits the activity of the beta-cells (28) These authors were careful to point out, however, that in a few instances evidence was obtained of hypersecretion of insulin from the pancreases of dogs given diabetogenic pituitary extracts for a short time

It was concluded by Haist Campbell and Best that the atrophy of the beta-cells in metahypophyseal diabetes was due to overstimulation of these cells leading to exhaustion through overwork and irreversible atrophy (23) The fact that fasting feeding fat diets or the administration of insulin prevented or reduced the injurious effects of the diabetogenic pituitary extracts favours this hypothesis It was observed by Allen that if partially

animals It therefore appears likely that in intact dogs the diabetogenic extracts create an increased metabolic demand for insulin and thus indirectly increase the rate of secretion of insulin by the pancreas

Similar results were obtained with purified growth hormone (14) Three depancreatized dogs, that had been maintained in good condition by use of insulin and a diet containing raw pancreas, were given relatively small doses of growth hormone (0 5 to 0 25 mg of DP per kg. body weight per day) The diet and dose of insulin remained constant. A great intensification of the diabetes occurred on giving the growth hormone The sugar excretion and urine volume increased, while lipaemia, increase in plasma non-protein nitrogen and ketonuria occurred Definite effects were noted within twenty-four hours and in two cases death in diabetic coma resulted after four and five days of injection Thus growth hormone acts on extrapancreatic tissues to increase the severity of diabetes

[illegible]

sity was great, it was not as lethal as in the depancreatized dogs. Evidently the presence of the pancreas protected the dogs from the diabetogenic effects of the growth hormone. Relatively small doses of the same growth preparation were also given to a metahypophyseal diabetic dog given the usual maintenance dose of insulin The result in this case was the same as in the depancreatized animals, that is, there were increases in hyperglycaemia, glycosuria, ketonuria and lipaemia Death due to diabetic coma occurred on the fourth day of injection When this dog (J) was in the normal state, much greater resistance to larger doses of growth hormone was found (13) Since the islet lesion is the chief functional defect so far recognized in meta- [illegible]

[illegible]

the presence of functional beta-cells and their activity in secreting

The studies of Milman, DeMoor and Lukens show that in the absence of insulin (hypophysectomized depancreatized cat) growth hormone does not cause retention of nitrogen and that insulin is essential for the protein anabolic effects of growth hormone (37) Depancreatized cats had impaired nitrogen retention despite the administration of insulin When depancreatized cats were given growth hormone and increasing amounts of insulin the retention of nitrogen was increased to the degree observed in normal animals The authors explain these results by the assumption that an increased secretion of insulin occurs in response to growth hormone in normal animals and that this increased demand for insulin may be sufficiently great to exceed the body's functional reserve

Salter and Best were able to induce growth in hypophysectomized rats by the administration of increasingly large doses of insulin (44) Growth in these animals was apparent from the increase in body weight and from the increases in the nitrogen and fat contents of the carcasses Insulin also caused a demonstrable increase in the width of the uncalcified cartilaginous disc of the tibia, similar to that found in hypophysectomized rats given growth hormone *It appears therefore that insulin can act* as a growth hormone in the absence of the pituitary gland, but

the absence of the pancreas Houssay, Biasotti and Rietti showed that diabetogenic pituitary extracts greatly intensify the severity of diabetes in depancreatized dogs (27a) Campbell, Keenan and Best found that when a diabetogenic pituitary fraction was administered to a depancreatized and to a metahypophyseal diabetic dog, the amounts of insulin required to control the diabetes were respectively 3 3-fold and 8-fold greater than those that were required before the extract was given (12) These results, and studies by other investigators, show that the diabetogenic material gives rise to increased demands for insulin by *extrapancreatic* tissues There is no reason to believe that these extrapancreatic effects are not exerted when the extracts are given to normal

organs of these dogs were not examined prior to giving the

It is therefore probable that the greatly enlarged and fatty liver found in these diabetic dogs after giving growth hormone can be ascribed to the purified hormone material We found that the administration of growth hormone to intact dogs increased the lipid contents of the livers, to a considerable extent in some cases and much less in others (11) The extent to which the lipid

sence of functional islet tissue tends to counteract the deposition of fat in the liver which occurs after growth hormone has been given

after pancreatectomy in the dog, the liver was much enlarged and very fatty and that this could be prevented by the administra-

was then reduced to one-half for two days, to one-quarter for two days and was then withdrawn completely for two to four days the animals being sacrificed on the seventh to the ninth day after the first reduction In these animals the liver was found to be enlarged and grossly fatty (Table II) Insulin thus prevents the rapid and massive deposition of fat in the livers of depancreatized dogs

The evidence indicates therefore, that, in diabetic dogs and in intact dogs, the increased deposition of lipid in the liver which

insulin Thus there are reasons for the deduction that in normal animals the growth hormone creates in the extrapancreatic tissues an increased demand for insulin and elicits an increased rate of production of insulin from the beta-cells of the islets of Langerhans This postulated stimulatory effect of growth hormone on the islets is indirect It appears that although growth hormone may, according to Anderson and Long, inhibit the insulin secretion of the rat pancreas directly (2), this inhibition must be overcome, in the early stages of injection of normal dogs by the more powerful indirect effects

EFFECTS OF GROWTH HORMONE ON LIVER LIPID

The changes that occurred in the lipid contents of the livers of these normal and diabetic dogs given growth hormone also

TABLE II

Liver weights and l p d contents

[illegible] diabetic dog J [illegible] age [illegible] and [illegible] th-
drawn for 4 days The diabetic dogs were given a diet containing raw pancreas

Treatment	Dog no	Body wt. (kg)	Liver Weight (g per kg body weight)	Liver Dry fat-free solids (g per kg body weight)	Liver Total lipid (g per kg body weight)
Depancreatized given insulin and growth hormone	D4	15 6	80 0	13 3	11.2
	D5	19 7	48.2	7 4	15 6
	D6	10 1	51 1	7 9	14 0
Metahypophyseal diabetic given insulin and growth hormone	J	10 1	59 5	7.4	21 3
Depancreatized insulin withdrawn	D1	10 8	44.1	5 8	15.4
	D2	7 7	54.5	8.4	17 9
	D3	5 8	72 5	9 5	29.4
Intact	N1	12 5	23 9	5.4	1 7
	N2	13 7	25 1	7 1	1 5
	N3	8 5	26 0	5 5	1.4
	N4	8 6	25 7	6 6	1 5
	N5	8 5	26 6	5 7	1 3
Means of N1 to N5			25.4	6 1	1 5

creates an increased metabolic demand for insulin which is countered in the intact animal by an increase in the rate of secretion of insulin by the islet cells

EFFECTS OF GROWTH HORMONE ON PLASMA FIBRINOGEN IN DIABETIC DOGS

It was observed that the administration of growth hormone [illegible]

to produce these effects When the administration of insulin to the depancreatized dogs was discontinued the erythrocyte sedimentation rate and the plasma fibrin levels were not altered significantly The inosaemia and increased erythrocyte sedimentation rate found after giving growth hormone are apparently therefore not due to relative deficiency of insulin, or to the effects thereof, such as hyperglycaemia and acidosis

According to Madden and Whipple, the fibrinogen of the plasma is produced by the liver (34) The growth hormone must increase the rate of addition of fibrinogen to the blood relative to the rate of removal Since after the administration of the hormone, the dry fat-free solids as well as the lipid content of the [illegible]

In depancreatized dogs we have observed that the sedimentation rate of the erythrocytes and the plasma fibrinogen concentrations are usually above normal Although these changes are not nearly as great as when growth hormone is given some disturbance is indicated It may be relevant to mention that Giannico and Marrazza find that in diabetes mellitus the plasma fibrinogen level may be elevated (20)

EFFECTS OF GROWTH HORMONE ON LEUCOCYTES IN DIABETIC ANIMALS

The administration of growth hormone produced a marked leucocytosis in the diabetic dogs given insulin This was due to a

occurs when growth hormone is given is due, in part at least, to a relative deficiency of insulin In the normal dogs also the increase in liver lipid caused by growth hormone appears to be due to deficiency of insulin, for at the time of sacrifice no granules

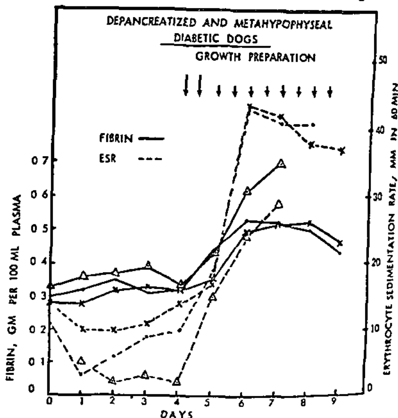


Fig 1

Effect of growth hormone administration on the blood fibrin content and erythrocyte sedimentation rate (ESR)

Depancreatized dogs (● *D5* × *D6*) and a metahypophyseal diabetic dog (Δ *J*) given growth hormone preparation D21P in dosage of 0 5 mg per kg per day on day 4 and one-half this dose thereafter The insulin dosage was unaltered. (from ref 14)

could be observed in the beta-cells of the pancreatic islets in two cases and only a few in another, while the extractable insulin contents were correspondingly low. These observations on liver lipids thus lead again to the conclusion that the growth hormone

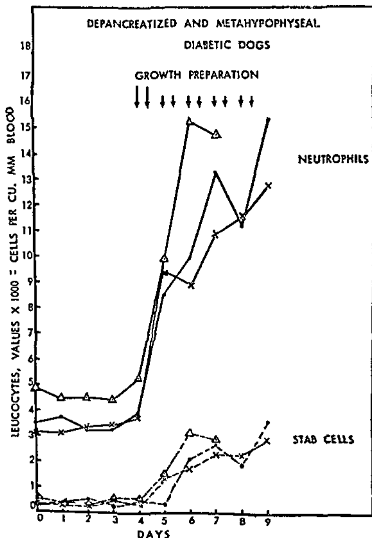


Fig 3

Effect of insul n dosage on the blood neutrophil and stab cell counts

Depancreatized dogs with insulin dosage gradually reduced then w thdrawn com pletely (from ref. 14)

marked increase in the number of neutrophils and a lesser increase in that of stab cells (Fig 2) A slight monocytosis was also noted The levels of the other types of white cells showed little change,

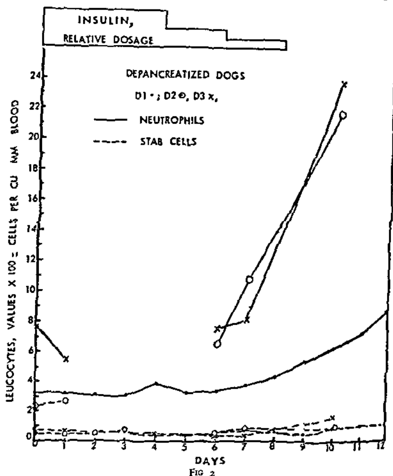


FIG 2

Effect of growth hormone administration on the blood neutrophil and stab cell counts
Depancreatized dogs (● D5 × D6) and a metahypophyseal diabetic dog (Δ J) given growth hormone preparation D21P in dosage of 0 5 mg per kg per day on day 4 and one-half this dose thereafter The insulin dosage was unaltered.

except for a slight decrease in the number of lymphocytes The eosinophil count was not lower

Cessation of insulin administration to depancreatized dogs

45 SAYERS, M A, SAYERS, G and WOODBURY, L A, *Endocrinology*, 42, 379 (1948)
46 SMITH, E L, BROWN, D M, FISHMAN, J B and WILHELMI, A E, *J biol Chem*, 177, 305 (1949)
47 WILHELMI, A E, FISHMAN, J B and RUSSELL, J A, *J biol Chem* 176, 735 (1948)
48 YOUNG, F G, *Lancet*, ii, 372 (1937)
49 YOUNG, F G, *Biochem J*, 32, 513 (1938)
50 YOUNG, F G, *Biochem J*, 39, 515 (1945)

DISCUSSION

YOUNG Have you made any observations on the amounts of glycogen in the livers of dogs with metahypophyseal diabetes, Dr Campbell? I am thinking of Professor Loubatières' results in this direction

CAMPBELL No

LOUBATIÈRES I have found that the level of hepatic glycogen in animals with metahypophyseal diabetes is greater than that in totally depancreatized animals but less than that in normal animals

HANSSEN Is the response shown by the neutrophils or fibrinogen an unspecific effect?

CAMPBELL Apparently not I did not mention that control dogs were treated with bovine plasma albumin and that no effect was observed The doses of growth hormone given simultaneously to [illegible]

diabetic animals?

CAMPBELL I know of no case

YOUNG Are there any cases in which there is a decrease in the intensity of diabetes?

CAMPBELL I do not think so

YOUNG In 1941 I observed an instance where the administration of a single dose of an [illegible]

19 GHOSH, B N, WOODBURY, D M and SAYERS, G, *Endocrinology*, 48, 631 (1951)
20 GIANNCO, O and MARRAZZA, P, *Policlinico*, 55, 573 (1948)
21 GREENSPAN, F S, LI, C H, SIMPSON, M E and EVANS, H M, *Endocrinology*, 45, 455 (1949)
22 HAIST, R E, *Physiol Rev*, 24, 409 (1944)
23 HAIST, R E, CAMPBELL, J and BEST, C H, *New Engl J Med* 223, 607 (1940)
24 HAM, A W and HAIST, R E, *Amer J Path*, 17, 787 (1941)
25 HOUSSAY, B A, *Endocrinology*, 30, 884 (1942)
26 HOUSSAY, B A and ANDERSON, E, *Endocrinology*, 45 627 (1949)
27 HOUSSAY, B A and BIASOTTI A, *C R Soc Biol*, *Paris*, 129 1259 (1938)
27a HOUSSAY B A, BIASOTTI, A and RIETTI, C T, *C R Soc Biol*, *Paris*, 111, 479 (1932)
28 HOUSSAY, B A, FOGLIA, V G, SMYTH, F S, RIETTI, C T and HOUSSAY, A B, *J exp Med*, 75, 547 (1942)
29 LI C H and EVANS, H M, *Recent Progr Hormone Res*, 3, 3 (1948)
30 LI, C H EVANS, H M and SIMPSON, M E, *J biol Chem*, 159, 353 (1945)
31 LI, C H and MOSKOWITZ, M, *J biol Chem*, 178, 203 (1949)
32 LOUBATIÈRES, A, *C R Soc Biol*, *Paris*, 132 384 (1939)
33 LYONS W R, *Proc Soc exp Biol*, *N Y*, 35, 645 (1937)
34 MADDEN, S C and WHIPPLE G H, *Physiol Rev*, 20, 194 (1940)
35 MARKS, H P and YOUNG, F G *Lancet*, 1, 493 (1940)
36 MARX, W SIMPSON, M E and EVANS, H M, *Endocrinology*, 30, 1 (1942)
37 MILMAN, A E, DEMOOR, P and LUKENS, F D W, *Amer J Physiol*, 166, 354 (1951)
38 MILMAN A E and RUSSELL, J A *Endocrinology*, 47, 114 (1950)
39 MUNROE J S and CHAIKOFF, L *Canad physiol Soc Proc*, p 45 (1952)
40 RABEN, M S and WESTERMEYER, V W, *Proc Soc exp Biol N Y*, 80 83 (1952)
41 REID E, *J Endocrin* 8, 50 (1952)
42 RICHARDSON, K C, *Proc roy Soc*, B, 128, 153 (1940)
43 RICHARDSON, K C and YOUNG, F G, *Lancet*, 1 1098 (1938)
44 SALTER, J M and BEST, C H, *Canad physiol Soc Proc*, p 57 (1952)

45 SAYERS, M A, SAYERS, G and WOODBURY, L A, *Endocrinology*, 42, 379 (1948)
46 SMITH, E L, BROWN, D M, FISHMAN, J B and WILHELMI, A E, *J biol Chem*, 177, 305 (1949)
47 WILHELMI, A E, FISHMAN, J B and RUSSELL, J A, *J biol Chem*, 176, 735 (1948)
48 YOUNG, F G, *Lancet*, ii, 372 (1937)
49 YOUNG, F G, *Biochem J*, 32, 513 (1938)
50 YOUNG, F G, *Biochem J*, 39 515 (1945)

DISCUSSION

YOUNG Have you made any observations on the amounts of glycogen in the livers of dogs with metahypophyseal diabetes, Dr Campbell? I am thinking of Professor Loubatières' results in this direction

CAMPBELL No

LOUBATIÈRES I have found that the level of hepatic glycogen in animals with metahypophyseal diabetes is greater than that in totally depancreatized animals but less than that in normal animals

HANSSEN Is the response shown by the neutrophils or fibrinogen an unspecific effect?

CAMPBELL Apparently not I did not mention that control dogs were treated with bovine plasma albumin and that no effect was observed The doses of growth hormone given simultaneously to the previously untreated dogs were small

YOUNG Is there any exception to the rule that growth hormone increases the severity of diabetes in depancreatized or metahypophyseal diabetic animals?

CAMPBELL I know of no case

YOUNG Are there any cases in which there is a decrease in the intensity of diabetes?

CAMPBELL I do not think so

YOUNG In 1941 I observed an instance where the administration of a single dose of [illegible]

spontaneously increased in severity with time, so the dog became less responsive to the glycosuria-diminishing action of the extract, although if the diabetes was then controlled by insulin, the extract again

19 GHOSH, B N, WOODBURY, D M and SAYERS, G, *Endocrinology*, 48, 631 (1951)
20 GIANNCO, O and MARRAZZA, P, *Policlinico*, 55, 573 (1948)
21 GREENSPAN, F S, LI, C H, SIMPSON, M E and EVANS H M *Endocrinology*, 45, 455 (1949)
22 HAIST, R E, *Physiol Rev*, 24, 409 (1944)
23 HAIST, R E, CAMPBELL, J and BEST, C H, *New Engl J Med* 223 607 (1940)
24 HAM, A W and HAIST, R E, *Amer J Path*, 17, 787 (1941)
25 HOUSSAY, B A, *Endocrinology*, 30, 884 (1942)
26 HOUSSAY, B A and ANDERSON, E, *Endocrinology*, 45 627 (1949)
27 HOUSSAY, B A and BIASOTTI, A, *C R Soc Biol*, *Paris*, 129 1259 (1938)
27a HOUSSAY, B A, BIASOTTI, A and RIETTI, C T, *C R Soc Biol*, *Paris*, 111, 479 (1932)
28 HOUSSAY, B A, FOGLIA, V G, SMYTH, F S, RIETTI, C T and HOUSSAY, A B, *J exp Med* 75, 547 (1942)
29 LI, C H and EVANS, H M, *Recent Progr Hormone Res*, 3, 3 (1948)
30 LI, C H, EVANS, H M and SIMPSON M E, *J biol Chem*, 159, 353 (1945)
31 LI, C H and MOSKOWITZ, M, *J biol Chem*, 178, 203 (1949)
32 LOUBATIÈRES, A, *C R Soc Biol*, *Paris*, 132 384 (1939)
33 LYONS W R, *Proc Soc exp Biol*, *N Y*, 35, 645 (1937)
34 MADDEN, S C and WHIPPLE, G H, *Physiol Rev*, 20 194 (1940)
35 MARKS, H P and YOUNG, F G *Lancet*, 1, 493 (1940)
36 MARX, W, SIMPSON, M E and EVANS, H M, *Endocrinology* 30, 1 (1942)
37 MILMAN, A E DEMOOR, P and LUKENS, F D W, *Amer J Physiol*, 166, 354 (1951)
38 MILMAN, A E and RUSSELL J A, *Endocrinology*, 47 114 (1950)
39 MUNROE, J S and CHAIKOFF, L, *Canad physiol Soc Proc*, p 45 (1952)
40 RABEN, M S and WESTERMEYER V W, *Proc Soc exp Biol*, *N Y*, 80, 83 (1952)
41 REID, E, *J Endocrin* 8, 50 (1952)
42 RICHARDSON, K C, *Proc roy Soc*, B, 128, 153 (1940)
43 RICHARDSON, K C and YOUNG, F G, *Lancet*, 1, 1098 (1938)
44 SALTER, J M and BEST, C H, *Canad physiol Soc Proc*, p 57 (1952)

LOUBATIÈRES I, too, am willing to admit that in the early stages little or no ketonuria is observed But subsequently it certainly develops, and may become intense I exclude, of course, certain exceptional and unexplained cases in which regression of diabetes mellitus may be observed!

Moreover, one of the observations which support the view that the metahypophyseal diabetes becomes more severe is the progressive increase in the value of the urinary D/N ratio observed when a diet composed exclusively of meat is fed. This ratio, very low at the beginning (1·2–1·3) may reach and exceed 2 8, or even 3 2, after several months

For these reasons, I maintain that, independently of other experi-

dogs we find a decrease in insulin requirement, in agreement with Professor Young We have also had cases in which there was little change It depends to some extent on the closeness of control over the animal If the animal is out of control the insulin requirement increases

YOUNG Our dogs were, we feel, under better control than many human diabetics

LAWRENCE We had three cases of balanced diabetes which were treated with Professor Young's growth-promoting extracts There was no change in the insulin requirement in two cases and in the third it rose by twenty to thirty per cent No pure growth hormone was used, however.

YOUNG It is surprising that the human being does not respond very readily to treatment with growth hormone

LONG Woodyate has reported that an alarming exacerbation of the diabetes occurred in a patient given a commercial extract of the anterior pituitary

depancreatized dogs Later, when metahypophyseal diabetes has existed for three or four months, fasting of long duration (three to four days) may slightly reduce the hyperglycaemia but does not suppress the glycosuria Diabetes mellitus is thus more severe in this latter stage Logically, therefore, the insulin requirement must be greater—at least during fasting

such as the intensity of glycosuria or the D/N ratio, for example

LOUBATIERES I am not convinced, but we are not using the same *criterion for estimating the severity of diabetes and establishing our* conclusions, and this probably accounts for our disagreement During your periods of control, your animals were fed and received injections of insulin During my periods of control, my animals were fasting and received no insulin I should point out that the differences in digestive absorption between Professor Young's dogs and totally depancreatized dogs (due to the suppression of external pancreatic secretion) do not in any way affect the interpretation of the facts which I put forward

Moreover, independently of the experiments which I carried out in 1939, Hedon is at present attempting to clarify this very point. He finds, as I did, that metahypophyseal diabetes increases in severity as it continues After three to four months of injections of anterior pituitary extract, ketosis becomes so marked that the animals die Such an effect does not seem to us to be compatible with any interpretation other than that which we suggest

YOUNG It depends how the intensity of the diabetes is assessed

LUKENS I agree that the severity of the diabetes may vary according to the method used in assessing it Any one criterion has definite limitations

LOUBATIERES Professor Young's view is that metahypophyseal diabetes does not increase in severity as it continues Dr Lukens will probably be able to give us his opinion on this point In your experience Dr Lukens, does metahypophyseal diabetes reach its maximum intensity at the outset?

LUKENS When the administration of pituitary extract is stopped the intensity of the metahypophyseal diabetes increases until about seventy to eighty-five per cent of the available dietary glucose is being excreted in the urine

YOUNG I agree A very large proportion of the available carbohydrate of the diet may be excreted in the early stages but there is no ketonuria Ketonuria is the danger

LOUBATIÈRES I, too, am willing to admit that in the early stages little or no ketonuria is observed But subsequently it certainly develops, and may become intense I exclude, of course certain exceptional and unexplained cases in which regression of diabetes mellitus may be observed?

Moreover, one of the observations which support the view that the metahypophyseal diabetes becomes more severe is the progressive increase in the value of the urinary D/N ratio observed when a diet composed exclusively of meat is fed This ratio very low at the beginning (1 2-1 3) may reach and exceed 2 8, or even 3·2 after several months

For these reasons, I maintain that, independently of other experimental conditions to be defined and discussed later a comparison of the insulin requirements of totally depancreatized dogs and of metahypophyseal dogs is valid only if one takes into account the stage of metahypophyseal diabetes at which the observations are made

LUKENS This rise in the D/N ratio certainly does occur when the disease is severe enough

YOUNG We have had animals with a D/N ratio of 2 5 (the normal is about 3 6) in which the insulin requirement was very much greater than in depancreatized dogs and which diminished on pancreatectomy

CAMPBELL Usually after depancreatizing metahypophyseal diabetic dogs we find a decrease in insulin requirement in agreement with Professor Young We have also had cases in which there was little change It depends to some extent on the closeness of control over the animal If the animal is out of control the insulin requirement increases

YOUNG Our dogs were we feel under better control than many human diabetics

LAWRENCE We had three cases of balanced diabetes which were treated with Professor Young's growth-promoting extracts There was no change in the insulin requirement in two cases and in the third it rose by twenty to thirty per cent No pure growth hormone was used, however

YOUNG It is surprising that the human being does not respond very readily to treatment with growth hormone

LONG Woodyate has reported that an alarming exacerbation of the diabetes occurred in a patient given a commercial extract of the anterior pituitary

INSULIN AND GROWTH HORMONE

By

S E DE JONGH

Pharmacologisch Laboratorium der Rijks-Universiteit, Boerhaavekwartier, Leiden, The Netherlands

INTRODUCTION

It is not an easy task for me to deal with the subject 'Insulin and Growth Hormone' at a conference which has the intention of covering the same subject in general The other contributors were more fortunate in being allotted special aspects of the field I must therefore apologize if I have occasionally intruded into their territory On the other hand, I may have misjudged the extent to which certain material is going to be dealt with by others, which may lead to undesirable omissions

The Effect of Growth Hormone on the Soft Tissues

sparing action In evaluating such a collaboration one must consider whether growth hormone primarily stimulates the growth of bone or rather that of soft tissue In the former case the growth of the soft tissues would be secondary to that of bone in the latter the growth of the skeletal structure should be considered as a special case of soft tissue growth, namely, that of the soft tissue of which the bone matrix consists

The circumstance that Dr Campbell has contributed a paper on growth hormone and protein metabolism has released me from the obligation of discussing in detail the question of the direct action of growth hormone on soft tissues, that is, the one which is not dependent on skeletal growth I shall report only the evidence provided by a single but instructive experiment. Boeré and Gaarenstroom administered impure growth hormone to rats several weeks after hypophysectomy (7) According to Freud *et al* , a bone plate' develops in the epiphyseal discs of the

absolute sense but it proved to hold for the circumstances and dosages used by Boere and Gaarenstroom No tail growth took place during the treatment, although there was a marked increase in body weight Muscles and intestines contributed proportionately to the weight increase, eliminating the possibility that the predominant effect on body weight was due to the deposition of fat—a supposition which is in any case most unlikely in view of all current experience in this field New protoplasm had obviously been formed Estimations of protein and water content revealed no major aberrations in the composition of the newly formed protoplasm (17)

The Protein-Sparing Effect of Growth Hormone

It is relevant for the development of our argument to distinguish between two possible ways in which the gain in body protein may take place It may either be due primarily to an increase in the manufacture of new protoplasm or be primarily the result of a protein-sparing action by growth hormone The latter possibility is supported by experiments which showed that nitrogen retention induced by growth hormone persists even when a protein-free diet is supplied (22) In face of the above evidence one feels justified in considering growth hormone as a hormone regulating protein metabolism. As to the mechanism of the action of growth hormone on protein metabolism, Russell and Capiello and Hoberman have argued that the effect is due to increased formation of protein, and not to decreased breakdown (24, 42), but the issue is not yet settled

Insulin and Protein Metabolism

If growth hormone is considered as a hormone affecting protein metabolism, there is the possibility of linking its action with that of insulin Mirsky, for instance, has provided evidence of the action of insulin on protein metabolism (39, 40) Insulin diminishes the amino-acid content of the blood of eviscerated dogs and accelerates the disappearance from the blood of injected amino-acids It was concluded from these results that insulin favours the formation of protein This action of insulin shall henceforth be referred to as the Mirsky effect.

This effect is not limited to the dog, but could also be demon-

strated in normal and even in hypophysectomized rats (46) The conclusion seems justified that it may occur in the absence of growth hormone and is therefore independent of it MacKay *et al* have shown that insulin induces retention of nitrogen (35), and following up these results, Maassen succeeded in enhancing [illegible]

fication for the view that insulin is figuring as a mediator of growth which has been brought about by some other mechanism.

THE PART PLAYED BY INSULIN IN THE GROWTH INDUCED BY GROWTH HORMONE

Diabetogenic Effect of Growth Hormone

As far as growth induced by growth hormone is concerned, strong experimental evidence in support of the conception just mentioned was obtained by Young (51, 52, 53, 54) in following up earlier experiments by Houssay (25) Young showed that an idiohypophyseal diabetic condition can be produced by the administration of hypophyseal extracts to adult dogs and cats The maintenance of this condition depends on the continuation of the treatment, but it may develop into permanent metahypophyseal diabetes which persists after cessation of treatment. This form of diabetes is characterized by damage to the beta-cells of the islets of Langerhans Young has stressed that attempts to separate the diabetogenic and the growth-promoting factors in pituitary extracts have always been unsuccessful. Thus Evans' earlier idea of an experimental diabetes induced by growth hormone (13) was *further elaborated* The diabetogenic effect of growth hormone has been amply confirmed (9, 10, 26) and has also been demonstrated by Young's group with the purified hormone

Enchancement of Insulin Production by Growth Hormone

It is generally agreed that the rise in the blood sugar level caused by growth hormone should result in an attempt by the pancreas to restore the equilibrium by producing more insulin. This effect is to be expected quite independently of the mechanism

by which growth hormone induces the rise in the blood sugar level Increased production of insulin during the idiohypophyseal phase, whether or not brought about by a rise in the blood sugar level, may be supported on various grounds

(i) Repeated injections of glucose may cause permanent damage to the beta-cells of the pancreas (12, 50) This effect is attributed to exhaustion of the pancreas, and suggests that the gland is very active in the preceding period

(ii) Young found that the sensitivity to insulin (as judged by its effect on the blood sugar level) is decreased in the idiohypophyseal phase. This low sensitivity did not prevent the animal from eventually recovering unless the dose of pituitary extract was increased This is thought to indicate an increase in the production of insulin.

(iii) Lukens and his associates found under similar circumstances that artificial lowering of the blood sugar level by insulin (31) or phlorrhizin (32) could prevent the development of metahypophyseal diabetes These results support the exhaustion theory mentioned above

(iv) Pituitary extracts are capable of enlarging the islets of Langerhans (6, 41) This again agrees with the idea of a hyperfunction of the islet tissue.

On the other hand, contrasting results were reported by Anderson and Long, who found with the perfused pancreas that addition of growth hormone to the perfusing fluid resulted in the release of less insulin (1) These results fall so little in line with the general impression that *in vivo* the amount of circulating insulin is increased, that one feels tempted to assume that Anderson's results may have been due to a focal overdosage of growth hormone

Therefore, in agreement with general opinion, the considerations which follow shall be governed by the assumption that growth hormone stimulates the release of insulin Young has put forward the attractive hypothesis that insulin through the Mirsky effect, mediates between growth hormone and growth This implies that growth hormone, like insulin, enriches the body protein store by means of an increased formation and not, or at least not primarily, by an inhibition of the breakdown of protein Young noticed that growth still continues during the

idiohypophyseal phase but stops during the metahypophyseal phase, and this course of events suggests that the extra insulin [illegible]

(i) Is insulin indispensable for growth, or merely stimulatory?
(ii) Is the increased production of insulin in fact the result of the rise in the blood sugar level during the idiohypophyseal phase, quite apart from the question as to what may be the cause of this rise? Or is another mechanism wholly or partly responsible?

These two questions will be discussed in turn

HOW DISPENSABLE IS INSULIN FOR GROWTH?

In view of the previous considerations, it will be necessary to attribute a liberal meaning to the term 'growth'. Accordingly, every sign of storage of protein will be considered as an indication that under these circumstances growth may occur

Growth Induced by Growth Hormone in the Absence of Insulin

A complete dependence of growth on the presence of insulin is suggested by Young's observations which have already been briefly summarized His puppies grew under treatment with growth hormone without contracting diabetes Adult dogs, however, became diabetic and the beta-cells of the islets of Langerhans were so severely damaged in the process that insulin production was not possible, these animals *did not grow* Puppies which were continuously injected with growth hormone grew until they had reached the adult age Thereafter, they sometimes became diabetic These phenomena may be understood as attempts by the pancreas to combat the idiohypophyseal rise of the blood sugar level by production of more insulin These attempts are successful in young animals with a resistant pancreas, but fail in older animals The rat, which is known to possess a particularly resistant pancreas, accordingly responds, even when adult, like [illegible]

that growth hormone can only induce growth when insulin is present.

idiohypophyseal phase but stops during the metahypophyseal phase, and this course of events suggests that the extra insulin released is of great importance for the inducement of growth

Of the many questions raised by these fundamental discoveries, two will be examined more closely

(i) Is insulin indispensable for growth, or merely stimulatory?

(ii) Is the increased production of insulin in fact the result of the rise in the blood sugar level during the idiohypophyseal phase, quite apart from the question as to what may be the cause of this rise? Or is another mechanism wholly or partly responsible?

These two questions will be discussed in turn

HOW DISPENSABLE IS INSULIN FOR GROWTH?

In view of the previous considerations, it will be necessary to attribute a liberal meaning to the term 'growth' Accordingly, every sign of storage of protein will be considered as an indication that under these circumstances growth may occur

Growth Induced by Growth Hormone in the Absence of Insulin

A complete dependence of growth on the presence of insulin is suggested by Young's observations which have already been briefly summarized His puppies grew under treatment with growth hormone without contracting diabetes Adult dogs, however, became diabetic and the beta-cells of the islets of Langerhans were so severely damaged in the process that insulin production was not possible, these animals *did not grow* Puppies which were continuously injected with growth hormone grew until they had reached the adult age Thereafter, they sometimes became diabetic These phenomena may be understood as attempts by the pancreas to combat the idiohypophyseal rise of the blood sugar level by production of more insulin These attempts are successful in young animals with a resistant pancreas, but fail in older animals The rat, which is known to possess a particularly resistant pancreas, accordingly responds, even when adult, like [illegible]

that growth hormone can only induce growth when insulin is present

Earlier experiments by Mirsky (39, 40) lead to similar conclusions pituitary extracts induced nitrogen retention in intact dogs, in pancreatectomized animals, however, the excretion of nitrogen was increased *In the former* case, a *latent* increase in nitrogen excretion (which cannot be caused by growth hormone) obviously had to be overcompensated for nitrogen retention to occur This retention of nitrogen, which at present we know to be due to growth hormone, *must* therefore have been even *more* pronounced than is suggested by the experimental figures

It is interesting in this connection to note that Gaebler and Robinson demonstrated that a relative nitrogen retention may also be induced by pituitary *extract in* pancreatectomized animals *provided* that the animals are treated with insulin (23) In contrast with these results are observations by Bennett and Li, and [illegible] nd de Jongh they found that nitrogen retention [illegible] vth hormone to rats [illegible] (5 48) Moreover, Gaarenstroom and de Jongh were able to produce actual growth by the injection of growth promoting extracts into hypophysectomized, alloxan diabetic rats (21) The risk inherent in experiments using alloxan *diabetic* animals, in view of the danger of the production of small amounts of insulin by residual undamaged tissue, was fully realized The observation, however by Anderson and Long that the perfused *pancreas* from alloxan diabetic animals failed to *produce insulin* (1) prevents me from leaving these experiments altogether outside the discussion Moreover, Milman and DeMoor could demonstrate an admittedly small retention of nitrogen following administration of growth hormone to pancreatectomized *cats* (37)

In view of these considerations we should like to uphold without reserve the opinion that insulin strongly supports the growth-promoting effect of growth hormone Complete dependence [illegible] action

[illegible] icy between the lack of growth of the diabetic dog in the metahypophyseal phase and the growth of the hypophysectomized alloxan diabetic rat possibly *exists only* superficially, even when the hypophysectomized alloxan diabetic rat is considered to be

deprived of insulin One must remember that hypophysectomy, although not restoring insulin to the body of the alloxan treated rat, in fact cures this animal of its diabetes (20) It is therefore possible that the difference in clinical condition between dog and rat may be of importance amongst the factors which determine whether administration of growth hormone will or will not

It is by no means certain that the growth-promoting effect of growth hormone is restricted to a single mechanism of interference with protein metabolism. If one assumes that more mechanisms than one are operating, the possibility arises that one *mechanism is more fully insulin-dependent than the others* That such a state of affairs may be the case is indicated by the following data concerning the influence of growth hormone on the amino-acid content of the blood

A Mechanism of Growth strongly Dependent on Insulin

It has been shown by various authors and under various circumstances that growth hormone and growth-promoting pituitary extracts are capable of eliciting a fall in the amino-acid content of the blood (15, 43) Newly eviscerated rats in which both the kidneys and adrenals have been removed or ligated show a considerable rise in the blood amino-acid level during an observation period of two hours This rise may be mitigated by the injection of growth-promoting pituitary extracts at the start of the experiment, the equilibrium amino-acid ⇌ protein *is apparently shifted further to the right* (47) *This effect* of growth hormone does not occur in newly eviscerated alloxan diabetic rats This suggests that the effect depends upon the presence of insulin remaining in the circulation shortly after the removal of the pancreas in such acute experiments *When the* experiment using eviscerated alloxan diabetic rats is repeated in the presence of small doses of parenterally administered insulin, pituitary extract is again effective (49) In these experiments the

a certain amount of insulin in the blood in normal animals It fails to occur in alloxan diabetic animals in the absence of insulin The importance of insulin for *this* action of growth hormone is therefore firmly established It is, however, equally certain that the effect does not depend on the production of a concomitant rise in the insulin level by growth hormone because such an increase cannot be expected to take place in eviscerated animals

A Mechanism of Growth less Dependent on Insulin

A curious effect was observed in rats which were deprived of their kidneys and adrenals but which possessed intact pancreases, livers and intestinal tracts In such animals, growth-promoting extracts induced an *increase* in the amino-acid content of the blood, whereas in the controls, presumably as a result of the activity of the liver, the amino-acid content remained practically constant during the observation period It seems unlikely that this reversal of the action of growth hormone was due to the presence of the pancreas or the intestinal tract It may, however, very well have been due to an impairment of some liver function in relation to amino-acid metabolism (presumably deamination) This phenomenon intrigued us because it occurred with undiminished intensity in alloxan diabetic rats. Quite apart from the question whether such an animal is insulin-free, it is apparent that growth hormone is capable of influencing protein metabolism in two different ways One of these does and the other does not involve the liver, and the latter effect is more insulin-dependent than the former

There are indications that the function of the liver in the situation just described is indeed connected with the breakdown of amino-acids Fraenkel-Conrat *et al* demonstrated that liver arginase is inhibited by growth hormone (14) They attributed this inhibition to a fall in the amino-acid level in the blood (and therefore in the liver) as a result of growth as such, that is, to a diminished supply of substrate for the enzyme Maassen, however, found that the rate of formation of ammonia from urea was lower in liver slices from rats which had been treated with growth-promoting pituitary extract, even when the amino-acid content of the blood (and therefore, presumably, the amount of

existence of an insulinotrophic effect appears to us a rather more cautious approach

Such an *effect on the beta-cells of the islets of* Langerhans has become the more interesting since Bornstein *et al* reported experiments showing that the diabetogenic action of growth hormone *may* also *be exerted via the pancreas* (8) Their experiments indicated that growth hormone increases the amount of glucagon secreted by the alpha-cells The idea that two mutually neutralizing actions, both operating on carbohydrate metabolism, would be situated in the same organ, is highly attractive The same experiments induced us to drop the familiar name 'pancreotrophic effect' and adopt the term 'insulinotrophic effect' to describe the action of growth hormone in lowering the blood sugar level The results of Bornstein are supported by the work of De Bodo *et al*, who found that growth hormone decreased the insulin sensitivity of hypophysectomized dogs (11) This effect could very well have been caused by the release of glucagon, resulting in the formation of glucose from liver glycogen

In the light of all these considerations, the answer to the second question may be summarized by the following statement the fact that the fall in the blood sugar level usually occurs after the administration of growth hormone strongly suggests that an increased secretion of insulin can be brought about by a process unconnected with the preceding diabetogenic effect of growth hormone

We accept the fact that growth hormone can exert an insulin-like *effect on the blood sugar level which is not connected* with its diabetogenic action We are inclined to interpret this insulin-like effect as an insulinotrophic one This interpretation is [illegible] the fact that the alternative explanation of an increase [illegible] growth hormone [illegible] experience

THEORETICAL CONSIDERATIONS

We shall now endeavour to coordinate the various effects of growth hormone on protein and carbohydrate metabolism into a system which makes biological sense The possibility that no

connection whatsoever may exist between the growth-promoting and hyperglycaemic effects of growth hormone can duly remain undiscussed [illegible]

[illegible] such a view compels one to assume that the body provides the necessary insulin through a latent, and sometimes manifest, hyperglycaemia. Seen from the angle of carbohydrate metabolism, the secretion of extra insulin has, in this interpretation, a compensatory function Growth induced by growth hormone would then occur at the risk of hyperglycaemia

Acceptance, however, of an insulinotrophic effect allows one to assume that the necessary insulin can be produced directly The so-called diabetogenic effect then appears to provide a useful means of compensation From a teleological point of view, the latter conception seems the more attractive because it implies that the production of insulin is directly dependent on carbohydrate metabolism It would appear unlikely that stimulation of insulin secretion would only be attained through a more or [illegible]

The fact that overdosage with growth hormone results in diabetes in various species supports either conception It is easy to understand that a maximal production of insulin may be insufficient to compensate a maximal antagonism of its effect on carbohydrate metabolism

Whichever of the two interpretations one may choose, in neither case does the diabetogenic action contribute to normal growth The name 'diabetogenic hormone' is therefore gradually losing its popularity It is desirable that physiological and not toxicological considerations should determine the nomenclature of hormones

GROWTH HORMONE AND FAT METABOLISM

The subject of this present paper does not permit me to give a detailed treatment of the influence of growth hormone on fat metabolism However, in order to provide a complete picture of events, I must make some brief remarks on this topic Growth occurs at the expense of available protein in the presence of an increased insulin concentration but of a more or less compensated, and therefore practically normal, carbohydrate metabolism. An additional source of energy is therefore required in order to compensate for the loss of that obtainable from the protein used up The only remaining source of supply of energy is fat, and the excess insulin present will hinder its utilization because insulin favours the formation of fat and its storage in the peripheral depots Mutually compensating effects of growth hormone are again to be expected and have actually been observed by various authors Bennett *et al* (3, 4) noted an increase in ketonaemia and ketonuria after injection of growth hormone After most

fat (55) Similar conclusions were reached by Li *et al* (29) A number of investigators found that administration of growth hormone resulted in deposition of fat in the liver which persisted for several days It was originally thought that the effect could be abolished by adrenalectomy, but it was later found to occur when this organ was still present (44, 45)

in vitro the rate of disappearance of fat from the liver tissue of

level of that of the treated animals It was found that liver slices from the treated animals when incubated *in vitro* lost significantly more fat than slices from the controls When compared with controls which had not been fed the additional amount of lard, the rats treated with growth hormone showed an increase in the initial liver fat content The assumption therefore seems justified

that increased mobilization of fat is not dependent on an increased breakdown of fat in the liver but rather is it an independent effect of treatment with growth hormone The short latent period makes it difficult to consider this effect as being independent of growth hormone and merely as a reaction to an impending shortage of calories, resulting from the exclusion of protein as a source of energy

RESERVATIONS

Many of the above considerations will need revision if Astwood's experiments can be confirmed. He claims to have [illegible]

it need not necessarily interfere with the picture of harmonious integration which has been drawn for body growth In such a case integration would, of course, be effected by three separate substances instead of a single one combining the three corresponding potencies

SUMMARY

To conclude, I should like to abandon, for the sake of clarity, all necessary reserve and caution, and give a schematic summary of our conception of the interrelationships of the various effects of growth hormone (using this term in its classical meaning)

1 Growth hormone secures equilibrium in the metabolism of carbohydrate by two different effects, both of which may have their site of action partly in the pancreas it increases the quantity of circulating insulin and in addition, neutralizes the effect of insulin on the blood sugar level Carbohydrate metabolism may proceed practically unchanged at a raised insulin level

2 This rise in the level of insulin favours at least one of the actions of growth hormone on protein metabolism, namely, the shift of the equilibrium amino-acid ⇌ protein to the right For the second effect, the inhibition of amino-acid breakdown in the liver, insulin is of less or perhaps of no importance.

3 The rise in the level of insulin impairs the utilization of fat as a source of energy in place of the protein which is lost This difficulty is combatted by a dual influence of growth hormone on fat metabolism peripheral mobilization and increased breakdown by the liver

4 This integration of the effects of growth hormone guarantees the fulfilment of caloric demands during its sparing action on proteins

REFERENCES

1 ANDERSON, E and LONG, J A, *Recent Progr Hormone Res*, 2, 217 (1948)
2 ASTWOOD, E B, personal communication
3 BENNETT, L L, GARCIA, J F and LI, C H, *Proc Soc exp Biol, N Y*, 68, 349 (1948)
4 BENNETT, L L, KREISS, R E and LI, C H, *Amer J Physiol*, 152, 210 (1948)
5 BENNETT, L L. and LI, C H, *J clin Endocrin*, 6, 462 (1946)
6 BEST, C H, CAMPBELL, J, HAIST, R. E and HAM, A W, *J Physiol*, 101, 17 (1942)
7 BOERE, J M and GAARENSTROOM, J H, *Versl gewone Vergad Akad Amst*, 52, 453 (1943)
8 BORNSTEIN, J, REID, E and YOUNG, F G, *Nature, Lond*, 168, 903 (1951)
9 CAMPBELL, J, DAVIDSON, I W F, SNAIR, W D and LEI, H P *Endocrinology*, 46, 272 (1950)
10 COTES, P. M, REID, E and YOUNG, F G, *Nature, Lond*, 164, 209 (1949)
11 DE BODO, R C., KURTZ, M, ANCOWITZ, A and KIANG, S P, *Proc Soc exp Biol, N Y*, 74, 524 (1950)
12 DOHAN, F C and LUKENS, F D W, *Endocrinology*, 42, 244 (1948)
13 EVANS, H M, MEYER, K., SIMPSON, M E and REICHERT, E L, *Proc Soc exp Biol, N Y*, 29 857 (1932)
14 FRAENKEL-CONRAT, H, SIMPSON, M E and EVANS, H M., *Amer J Physiol*, 138, 439 (1943)
15 FRAENKEL-CONRAT, J, FRAENKEL-CONRAT, H and EVANS, H M, *Amer J Physiol*, 137, 200 (1942)
16 FREUD, J, LEVIE, J A and KROON, D B, *J Endocrin*, 1, 57 (1939)
17 GAARENSTROOM, J A, *Proc Acad Sci Amst*, 49, 265 (1946)

18 GAARENSTROOM, J H, HUBLÉ, J and DE JONGH, S E, *Acta endocr, Copenhagen*, 2, 317 (1949)
19 GAARENSTROOM, J H, HUBLÉ, J and DE JONGH, S E, *J Endocrin*, 6, 71 (1949)
20 GAARENSTROOM, J H and DE JONGH, S E, *Acta brev neerl Physiol*, 14, 28 (1946)
21 GAARENSTROOM, J H and DE JONGH, S E, *Proc Acad Sci Amst*, 51, 166 (1948)
22 GAARENSTROOM, J H and KRET, A, *Proc Acad Sci Amst*, 48, 537 (1945)
23 GAEBLER, O H and ROBINSON, A R, *Endocrinology*, 30, 627 (1942)
24 HOBERMAN, H D, *Yale J Biol Med*, 22, 341 (1950)
25 HOUSSAY, B A, *Endocrinology*, 30, 884 (1942) and earlier papers
26 HOUSSAY, B A and ANDERSON, E, *Endocrinology*, 45 629 (1949)
27 KOCHAKIAN, C D and STETTNER, C E, *Amer J Physiol*, 155, 262 (1948)
28 KURTZ, M, DE BODO, R C, KIANG, S P and ANCOWITZ, A, *Proc Soc exp Biol, N Y*, 76, 21 (1951)
29 LI, C H, SIMPSON, M E and EVANS, H M, *Endocrinology*, 44, 71 (1949)
30 LONG, C N H, *Diabetes*, 1, 3 (1952)
31 LUKENS, F D W and DOHAN, F C, *Endocrinology*, 30, 175 (1942)
32 LUKENS, F D W, DOHAN, F C and WOLCOTT, M W, *Endocrinology*, 32, 475 (1943)
33 MAASSEN, A P, *Proc Acad Sci Amst*, 54, 160 (1951)
34 MAASSEN, A P, *Arch int Pharmacodyn* 88, 434 (1952)
35 MACKAY, E M, BARNES, R H and BERGMAN, H C, *Amer J Physiol*, 126, 155 (1939)
36 MARX, W, HERRING, V V and EVANS, H M, *Amer J Physiol*, 141, 88 (1944)
37 MILMAN, A E and DEMOOR, P, *Fed Proc*, 9, 90 (1950)
38 MILMAN, A E and RUSSELL, J A, *Fed Proc*, 8, 111 (1949)
39 MIRSKY, I A, *Amer J Physiol*, 124, 569 (1938)
40 MIRSKY, I A, *Endocrinology*, 25, 52 (1939)
41 RICHARDSON, K C and YOUNG, F G, *J Physiol*, 91, 352 (1937)
42 RUSSELL, J A and [illegible] 9)
43 S [illegible] 5)
44 S [illegible]
45 WEIL, R and ROSS, S, *Endocrinology*, 45, 207 (1949)
46 VAN WIERINGEN, G, *Acta brev neerl Physiol*, 14, 74 (1946)

3 The rise in the level of insulin impairs the utilization of fat as a source of energy in place of the protein which is lost This difficulty is combatted by a dual influence of growth hormone on fat metabolism peripheral mobilization and increased breakdown by the liver

4 This integration of the effects of growth hormone guarantees the fulfilment of caloric demands during its sparing action on proteins

REFERENCES

1 ANDERSON, E and LONG, J A, *Recent Progr Hormone Res*, 2, 217 (1948)
2 ASTWOOD, E B, personal communication
3 BENNETT, L L, GARCIA, J F and LI, C H, *Proc Soc exp Biol, N Y*, 68, 349 (1948)
4 BENNETT, L L, KREISS, R E and LI, C H, *Amer J Physiol*, 152 210 (1948)
5 BENNETT, L L and LI, C H, *J clin Endocrin*, 6, 462 (1946)
6 BEST, C H, CAMPBELL, J, HAIST, R E and HAM, A W, *J Physiol*, 101, 17 (1942)
7 BOERE, J M and GAARENSTROOM, J H, *Versl gewone Vergad Akad Amst*, 52, 453 (1943)
8 BORNSTEIN, J, REID, E and YOUNG, F G, *Nature, Lond*, 168 903 (1951)
9 CAMPBELL, J, DAVIDSON, I W F, SNAIR, W D and LEI, H P, *Endocrinology*, 46, 272 (1950)
10 COTES, P M, REID E and YOUNG, F G, *Nature, Lond*, 164, 209 (1949)
11 DE BODO, R C, KURTZ, M, ANCOWITZ, A and KIANG, S P, *Proc Soc exp Biol, N Y*, 74, 524 (1950)
12 DOHAN F C and LUKENS, F D W, *Endocrinology*, 42, 244 (1948)
13 EVANS, H M, MEYER K SIMPSON M E and REICHERT, E L., *Proc Soc exp Biol, N Y*, 29 857 (1932)
14 FRAENKEL-CONRAT, H, SIMPSON, M E and EVANS, H M, *Amer J Physiol*, 138, 439 (1943)
15 FRAENKEL-CONRAT, J, FRAENKEL-CONRAT, H and EVANS, H. M, *Amer J Physiol*, 137, 200 (1942)
16 FREUD, J, LEVIE, J A and KROON, D B, *J Endocrin*, 1, 57 (1939)
17 GAARENSTROOM, J A, *Proc Acad Sci Amst*, 49, 265 (1946)

18 GAARENSTROOM, J H, HUBLÉ, J and DE JONGH, S E, *Acta endocr, Copenhagen*, 2, 317 (1949)
19 GAARENSTROOM, J H, HUBLE, J and DE JONGH, S E, *J Endocrin*, 6, 71 (1949)
20 GAARENSTROOM, J H and DE JONGH, S E, *Acta brev neerl Physiol*, 14, 28 (1946)
21 GAARENSTROOM, J H and DE JONGH, S E, *Proc Acad Sci Amst*, 51, 166 (1948)
22 GAARENSTROOM, J H and KRET, A, *Proc Acad Sci Amst*, 48, 537 (1945)
23 GAEBLER, O H and ROBINSON, A R, *Endocrinology*, 30 627 (1942)
24 HOBERMAN, H. D, *Yale J Biol Med*, 22, 341 (1950)
25 HOUSSAY, B A, *Endocrinology*, 30, 884 (1942) and earlier papers
26 HOUSSAY, B A and ANDERSON, E, *Endocrinology*, 45, 629 (1949)
27 KOCHAKIAN, C D and STETTNER C E, *Amer J Physiol*, 155, 262 (1948)
28 KURTZ, M, DE BODO, R C, KIANG, S P and ANCOWITZ, A, *Proc Soc exp Biol, N Y*, 76 21 (1951)
29 LI, C H SIMPSON, M E and EVANS, H M, *Endocrinology*, 44 71 (1949)
30 LONG, C N H, *Diabetes*, 1, 3 (1952)
31 LUKENS, F D W and DOHAN, F C, *Endocrinology*, 30 175 (1942)
32 LUKENS, F D W, DOHAN, F C and WOLCOTT, M W, *Endocrinology* 32, 475 (1943)
33 MAASSEN, A P, *Proc Acad Sci Amst*, 54, 160 (1951)
34 MAASSEN, A P, *Arch int Pharmacodyn* 88, 434 (1952)
35 MACKAY, E M, BARNES, R. H and BEGMAN, H. C, *Amer J Physiol*, 126, 155 (1939)
36 MARX, W, HERRING, V V and EVANS, H M, *Amer J Physiol*, 141, 88 (1944)
37 MILMAN, A. E and DEMOOR, P, *Fed Proc*, 9, 90 (1950)
38 MILMAN, A E and RUSSELL, J A, *Fed Proc*, 8, 111 (1949)
39 MIRSKY, I A, *Amer J Physiol*, 124, 569 (1938)
40 MIRSKY, I A, *Endocrinology*, 25, 52 (1939)
41 RICHARDSON, K C and YOUNG, F G, *J Physiol*, 91, 352 (1937)
42 RUSSELL, J A and CAPIELLO, M, *Endocrinology* 44 520 (1949)
43 SCHAEFFER, N K and LEE, M, *J biol Chem*, 108, 355 (1935)
44 SZEGO, C M and WHITE, A, *Endocrinology*, 44, 150 (1949)
45 WEIL, R. and ROSS, S, *Endocrinology*, 45, 207 (1949)
46 VAN WIERINGEN, G, *Acta brev neerl Physiol*, 14, 74 (1946)

47 VAN WIERINGEN, G , *Arch int Pharmacodyn* , 36, 450 (1948)
48 VAN WIERINGEN, G and DE JONGH, S E , *Proc Acad Sci Amst* , 52, 835 (1949)
49 WIJNANS, M and DE JONGH, S E , *Acta physiol pharm neerl* , 2, 74 (1951)
50 WOERNER, C A , *Anat Rec* , 71, 33 (1938)
51 YOUNG, F G , *Lancet*, ii, 372 (1937)
52 YOUNG, F G , *J Endocrin* , 1, 339 (1939)
53 YOUNG, F G , *Brit Med J* , ii, 897 (1941)
54 YOUNG, F G , *Biochem J* , 39, 515 (1945)
55 YOUNG, F G , *J clin Endocrin* , 10, 824 (1950)

DISCUSSION

YOUNG I entirely agree that the name 'diabetogenic hormone' is not a good one to use, since the production of diabetes is obviously not a physiological result of the secretion of the hormone

I should like to refer briefly to some work carried out in our labora-

e

response of the blood sugar level to blood from other animals Briefly, our findings were as follows

(i) Growth hormone had little direct effect on these ADHA animals However, if blood from such an animal treated with growth hormone was taken twenty-four hours later and injected into a second ADHA rat, a rise in the blood sugar level occurred in the recipient animal One possible explanation of this effect is that the growth hormone stimulated the production of glucagon in the donor rat

(ii) Normal cats were treated with growth hormone for five or six days, so that a state of idiohypophyseal diabetes was produced and blood was then taken from the pancreatico-duodenal vein and injected into ADHA rats This provoked hyperglycaemia However, if the blood was taken after only two days, a slight (*perhaps not significant*) hypoglycaemia was produced It seems possible that an extra secretion of insulin by the pancreas occurred first of all and that this was later counteracted by the secretion of an excess of glucagon

vel

ent

lin

in the peripheral blood although none could be detected in the pan-

creatico-duodenal blood Alternatively, it is possible that insulin was present in the pancreatico-duodenal blood, but that the same amount in the peripheral blood produced a greater effect because here the insulin antagonist was no longer effective for some unknown reason

These results are in keeping with the view that the hypoglycaemic-glycogenolytic factor is liberated from the pancreas—and perhaps also some insulin at the same time—under the influence of growth hormone This idea will perhaps explain the results obtained by Anderson which gave no evidence of the liberation of insulin by the perfused rat pancreas when growth hormone was added to the perfusing fluid

DE JONGH I am in complete agreement with these views

RUSSELL Some work carried out recently in our laboratory on the effects of growth hormone on the metabolism of nitrogen in eviscerated or in nephrectomized rats has in part confirmed some of the work described by Professor de Jongh We carried out three experiments

(i) Using eviscerated rats, we followed the change in the blood or plasma amino-nitrogen level with time For the first two hours there was very little change After that, the plasma amino-nitrogen level began to rise steadily and this increase could be partially inhibited by insulin. In our hands, growth hormone alone had no effect on the release of amino-nitrogen into the plasma, but if given with insulin it produced a further inhibition Since the doses of insulin needed were rather large, the precise significance of this synergism is not clear

(ii) In another series of experiments, casein hydrolysate was given *intravenously to eviscerated rats, beginning about two hours after the* operation The plasma amino-nitrogen level, elevated by the infusion, fell to a stable level ½ to 1 hour after the injection and then began to *rise on a course parallel to, but at a higher level than, that in the animals* not given amino-acids If growth hormone was given to the animals before the injection of the hydrolysate, the intermediate level to which the plasma amino-nitrogen content fell was distinctly lowered From this, it appears that if the supply of amino-acids is sufficient growth hormone may induce retention of nitrogen in the peripheral tissues in the absence of the liver and also in the absence of all but traces of insulin

(iii) We studied the accumulation of urea in the blood of nephrectomized rats to obtain a measure of nitrogen catabolism Growth hormone alone had little effect on the rate of urea formation in the fasting animal When casein hydrolysate was given intravenously however, the rate of urea formation, much increased over that seen in the fasting controls, was depressed markedly by the prior administration

of growth hormone The free amino-acid concentration in either the plasma or the liver was not increased, but rather diminished somewhat, by the hormone *The effect does not seem, therefore, to* have been due to a reduced rate of hepatic clearance of amino-acids or to restriction of deaminase activity or urea formation *per se* Insulin in moderate amount, without extra glucose, had no effect on the rate of urea formation, either with or without administered amino-acids

These results suggest that the effect of growth hormone is principally on the uptake of exogenous amino-acids rather than on the breakdown of tissue protein and that a most important determinant of whether or not an effect of the hormone may be observed is the availability of extra nitrogen The liver may be a site of action of the hormone but its *presence is not essential for expression of activity*
[illegible]

of insulin on nitrogen metabolism is seen, it is when extra supplies of carbohydrate are available Growth hormone, on the other hand, can act in the absence of added carbohydrate, energy presumably being obtained at the expense of fat or ketone bodies

BEST I think there must almost always be a small amount of insulin remaining in alloxan diabetic animals There should be no insulin remaining [illegible]
however, providing t [illegible]
found that hypophy[illegible]
carbohydrate diet, would 'grow' when given small doses of slow-acting insulin The animals increased in weight by up to 11 g per [illegible] for periods of five
[illegible] in hypophys-
[illegible] inistration of
insulin alone if care is taken to maintain the blood sugar level during the experiment There was an increase in weight in most of the organs and retention of nitrogen The tails increased in length (in one case by as much as 1 5 cm) and the tibiae also definitely responded There was, therefore, an indication of skeletal growth, although this may prove to be less in amount than that induced by growth hormone *This was a highly unphysiological experiment, but the results are* very interesting because they show that insulin alone can produce a very profound effect My colleague Mr James Salter has been largely *responsible for* this work

DE JONGH A few years ago we carried out some experiments similar to those described by Professor Best Although all the animals died, as did Professor Best's if the experiment was not done very care-

fully, there was a significant difference between the falls in weight in the two groups of animals

LONG: The demonstration of the growth-promoting effect of pituitary extracts was originally made with plateaued female rats. Does insulin have a similar effect on the plateaued normal female rat?

BEST: This should be much easier to study and we will do it.

DE JONGH: It has been done in young rats.

BEST: It was not done with a reduced or fixed food intake.

RUSSELL: The hypophysectomized rat will increase in weight just with feeding. Therefore one must have data on the composition of the diet.

BEST: This is available. There was a large retention of nitrogen.

RUSSELL: Samuels force-fed hypophysectomized animals and found that they gained in weight, but that it was in fat and not in nitrogen retention.

YOUNG: Some preliminary experiments by R. H. Smith in our laboratory have shown that some growth occurs when glucagon and insulin are simultaneously administered to hypophysectomized rats, though the evidence is not yet complete. In our experiments the controls were given the same amount of food. If the conclusions of Bornstein, Reid and Young are correct, then one might well expect the effect of growth hormone to be duplicated by a mixture of insulin and glucagon.

RUSSELL: There are differences in the effects of insulin and growth hormone. Insulin has little or no effect unless glucose is given. It may therefore be making carbohydrate available. Growth hormone, however, does not require the addition of carbohydrate for its effect to be observed.

BEST: It has been found that treatment of a depancreatized dog with growth hormone, although it raised the blood sugar level, did not cause retention of nitrogen. If insulin was also given, however, nitrogen retention occurred.

RUSSELL: The administration of glucose made no difference in our experiments with growth hormone.

LUKENS: It seems that different responses are obtained in the diabetic animal according to whether or not insulin is present.

CARL CORI: I should like to mention again some unpublished experiments by Krahl which I reported at the *Ciba Foundation Colloquium on Hormonal Factors in Carbohydrate Metabolism*.

He studied the incorporation of [^{14}C]glycine into glutathione and protein in liver slices. There was a rapid incorporation into both components in slices taken from the normal animal. When the animal had been fasted for twenty-four hours, the rate of incorporation into

indeed Glucose produced some stimulation but the rate was not normal unless insulin was added as well In the presence of insulin alone there was no measurable incorporation Therefore, in the tissue from the diabetic animal, the effect is dependent upon the presence of both carbohydrate and insulin

YOUNG Professor Best said earlier on that extra insulin may not be necessary for the activity of growth hormone to be expressed but that this does not mean that extra insulin is not secreted under the influence of growth hormone under physiological conditions With this I am in complete agreement

NITROGEN RETENTION AND THE ACTION OF INSULIN

By

F D W. LUKENS

The George S Cox Medical Research Institute, University of Pennsylvania, Philadelphia, Pennsylvania, U S A

INTRODUCTION

Since the action of insulin is unknown, except by its various physiological effects, 'The Influence on Nitrogen Retention' might more appropriately express the topic of this paper. For purposes of this discussion, the term nitrogen retention should be defined The retention of nitrogen commonly refers to the nitrogen balance of the organism, which in turn measures the extent of protein anabolism or catabolism of the body as a whole The nitrogen balance is negative in starvation or stress, it is in fairly stable equilibrium in the adequately fed adult, and the balance becomes positive under the influence of growth hormone The role of insulin in nitrogen retention must be viewed against the background of these various conditions Furthermore, it is appropriate to recall some of the methods used to study the nitrogen balance of the body These methods include the usual metabolic measurements, in which dietary, urinary and faecal nitrogen are determined Other methods are body growth, measured as gain in weight or in length (*i e*, size) or both, gain or loss of weight under certain circumstances, changes in the composition of the carcass, especially in its protein or nitrogen content, changes in the rate of accumulation of blood nitrogen in nephrectomized animals, and more recently, the retention of nitrogen has been followed by the incorporation of isotopically labelled amino-acids All of these methods for the study of nitrogen metabolism have been employed in experiments in which the important metabolic hormones have been removed or administered under different conditions

From this large amount of material I shall try to select the principal observations which pertain to the influence of insulin on nitrogen (protein) metabolism As the parts played by insulin and growth hormone are particularly considered, two things must be kept in mind first, the fact that the effect on nitrogen

indeed Glucose produced some stimulation but the rate was not normal unless insulin was added as well In the presence of insulin alone there was no measurable incorporation Therefore, in the tissue from the diabetic animal, the effect is dependent upon the presence of both carbohydrate and insulin

YOUNG Professor Best said earlier on that extra insulin may not be necessary for the activity of growth hormone to be expressed but that this does not mean that extra insulin is not secreted under the influence of growth hormone under physiological conditions With this I am in complete agreement

NITROGEN RETENTION AND THE ACTION OF INSULIN

By

F D W LUKENS

The George S Cox Medical Research Institute, University of Pennsylvania, Philadelphia, Pennsylvania, U S A

INTRODUCTION

Since the action of insulin is unknown, except by its various physiological effects, 'The Influence on Nitrogen Retention' might more appropriately express the topic of this paper For purposes of this discussion, the term nitrogen retention should be defined. The retention of nitrogen commonly refers to the nitrogen balance of the organism, which in turn measures the extent of protein anabolism or catabolism of the body as a whole The nitrogen balance is negative in starvation or stress, it is in fairly stable equilibrium in the adequately fed adult, and the balance becomes positive under the influence of growth hormone The role of insulin in nitrogen retention must be viewed against the background of these various conditions Furthermore, it is appropriate to recall some of the methods used to study the nitrogen balance of the body These methods include the usual metabolic measurements, in which dietary, urinary and faecal nitrogen are determined Other methods are body growth measured as gain in weight or in length (i e , size) or both, gain or loss of weight under certain circumstances, changes in the composition of the carcass, especially in its protein or nitrogen content, changes in the rate of accumulation of blood nitrogen in nephrectomized animals, and, more recently, the retention of nitrogen has been followed by the incorporation of isotopically labelled amino-acids All of these methods for the study of nitrogen metabolism have been employed in experiments in which the important metabolic hormones have been removed or administered under different conditions.

From this large amount of material I shall try to select the principal observations which pertain to the influence of insulin on nitrogen (protein) metabolism As the parts played by insulin and growth hormone are particularly considered two things must be kept in mind first, the fact that the effect on nitrogen

xistence of an insulinotro
autious approach

Such an effect on the be
ecome the more interes
xperiments showing that
ormone may also be e
xperiments indicated that
f glucagon secreted by
utually neutralizing acti
etabolism, would be sit
tractive The same exper
ame 'pancreotrophic effec
fect' to describe the actio
lood sugar level The res
ork of De Bodo *et al*, v
eased the insulin sensitiv
his effect could very wel
lucagon, resulting in the f
n

In the light of all these co
estion may be summari
ct that the fall in the blo
ministration of growth
creased secretion of insul
connected with the prec
rmone

We accept the fact that g
e effect on the blood sug
diabetogenic action We
e effect as an insulino
dorsed by the fact that the
insulin sensitivity follow
pears highly unlikely in t

THEORETIC

We shall now endeavou
owth hormone on prote
system which makes biol

connection whatsoever may exist between the growth-promoting and hyperglycaemic effects of growth hormone can duly remain undiscussed

secures an increase in its insulin supply The insulin thus liberated may assist growth, as has been discussed earlier

Further elaboration of this train of thought depends on one's willingness to accept the idea that there can be an insulinotrophic effect of the kind which I have just mentioned Refutation of such a view compels one to assume that the body provides the

pensatory function Growth induced by growth hormone would then occur at the risk of hyperglycaemia

Acceptance, however, of an insulinotrophic effect allows one to assume that the necessary insulin can be produced directly The so-called diabetogenic effect then appears to provide a useful means of compensation From a teleological point of view, the latter conception seems the more attractive because it implies that the production of insulin is directly dependent on carbohydrate metabolism It would appear unlikely that stimulation of insulin secretion would only be attained through a more or le

a

o

to compensate a maximal antagonism of its effect on carbohydrate metabolism

Whichever of the two interpretations one may choose, in neither case does the diabetogenic action contribute to normal growth The name 'diabetogenic hormone' is therefore gradually losing its popularity It is desirable that physiological and not toxicological considerations should determine the nomenclature of hormones

existence of an insulinotrophic effect appears to us a rather more cautious approach

Such an effect on the beta-cells of the islets of *Langerhans* has become the more interesting since Bornstein *et al.* reported experiments showing that the diabetogenic action of growth hormone may also be exerted via the pancreas (8) Their experiments indicated that growth hormone increases the amount of glucagon secreted by the alpha-cells. The idea that two mutually neutralizing actions, both operating on carbohydrate metabolism, would be situated in the same organ, is highly attractive. The same experiments induced us to drop the familiar name 'pancreotrophic effect' and adopt the term 'insulinotrophic effect' to describe the action of growth hormone in lowering the blood sugar level The results of Bornstein are supported by the work of De Bodo *et al* [illegible] hormone de-creased the insulin sensi [illegible] This effect could very [illegible] glucagon, resulting in the formation of glucose from liver glycogen.

In the light of all these considerations, the answer to the second question may be summarized by the following statement. the fact that the fall in the blood sugar level usually occurs after the administration of growth hormone strongly suggests that an increased secretion of insulin can be brought about by a process unconnected with the preceding diabetogenic effect of growth hormone. [illegible] as an insulin- [illegible] h [illegible] like effect as an insulinotropic one. [illegible] s endorsed by the fact that the alternative explanation of an increase in insulin sensitivity following treatment with growth hormone appears highly unlikely in the light of general experience.

THEORETICAL CONSIDERATIONS

We shall now endeavour to coordinate the various effects of growth hormone on protein and carbohydrate metabolism into a system which makes biological sense. The possibility that no

connection whatsoever may exist between the growth-promoting and hyperglycaemic effects of growth hormone can duly remain undiscussed
[illegible]

secures an increase in its insulin supply The insulin thus liberated may assist growth as has been discussed earlier
[illegible]

such a view compels one to assume that the body provides the necessary insulin through a latent and sometimes manifest hyperglycaemia Seen from the angle of carbohydrate metabolism, the secretion of extra insulin has in this interpretation a compensatory function Growth induced by growth hormone would then occur at the risk of hyperglycaemia

Acceptance, however of an insulinotrophic effect allows one to assume that the necessary insulin can be produced directly The so-called diabetogenic effect then appears to provide a useful means of compensation From a teleological point of view, the latter conception seems the more attractive because it implies that the production of insulin is directly dependent on carbohydrate metabolism. It would appear unlikely that stimulation of insulin secretion would only be attained through a more or
[illegible]

The fact that overdosage with growth hormone results in diabetes in various species supports either conception. It is easy to understand that a maximal production of insulin may be insufficient to compensate a maximal antagonism of its effect on carbohydrate metabolism

Whichever of the two interpretations one may choose, in neither case does the diabetogenic action contribute to normal growth The name diabetogenic hormone' is therefore gradually losing its popularity It is desirable that physiological and not toxicological considerations should determine the nomenclature of hormones

GROWTH HORMONE AND FAT METABOLISM

The subject of this present paper does not permit me to give a detailed treatment of the influence of growth hormone on fat metabolism However, in order to provide a complete picture of events, I must make some brief remarks on this topic. Growth occurs at the expense of available protein in the presence of an increased insulin concentration but of a more or less compensated, and therefore practically normal, carbohydrate metabolism. An additional source of energy is therefore required in order to compensate for the loss of that obtainable from the protein used up The only remaining source of supply of energy is fat, and the excess insulin present will hinder its utilization because insulin favours the formation of fat and its storage in the peripheral depots Mutually compensating effects of growth hormone are again to be expected and have actually been observed by various authors Bennett *et al* (3, 4) noted an increase in ketonaemia and ketonuria after injection of growth hormone After most ingenious and carefully conducted experiments concerning changes in fat metabolism Young reached the conclusion that growth hormone does indeed render protein available at the expense of fat (55) Similar conclusions were reached by Li *et al* (29) A number of investigators found that administration of growth hormone resulted in deposition of fat in the liver which persisted for several days It was originally thought that the effect could [illegible] was later found to occur

[illegible]

does active breakdown of fat also take place? Maassen studied *in vitro* the rate of disappearance of fat from the liver tissue of rats which had been given a single injection of growth hormone sixteen hours previously (10) The untreated controls received an additional supply of lard to increase their initial liver fat to the level of that of the treated animals It was found that liver slices from the treated animals when incubated *in vitro* lost significantly more fat than slices from the controls When compared with controls which had not been fed the additional amount of lard, the rats treated with growth hormone showed an increase in the initial liver fat content The assumption therefore seems justified

that increased mobilization of fat is not dependent on an increased breakdown of fat in the liver but rather is it an independent effect of treatment with growth hormone. The short latent period makes it difficult to consider this effect as being independent of growth hormone and merely as a reaction to an impending shortage of calories, resulting from the exclusion of protein as a source of energy.

RESERVATIONS

Many of the above considerations will need revision if Astwood's experiments can be confirmed. He claims to have isolated preparations of growth hormone which lack effects on carbohydrate and fat metabolism (2). Moreover, he reports that he has separated the 'diabetogenic' hormone from one having an effect on fat metabolism. The repercussions of this important discovery, if it is confirmed, would be tremendous. However, it need not necessarily interfere with the picture of harmonious integration which has been drawn for body growth. In such a case integration would, of course, be effected by three separate substances instead of a single one combining the three corresponding potencies.

SUMMARY

To conclude, I should like to abandon, for the sake of clarity, all necessary reserve and caution, and give a schematic summary of our conception of the interrelationships of the various effects of growth hormone (using this term in its classical meaning).

1 Growth hormone secures equilibrium in the metabolism of carbohydrate by two different effects, both of which may have their site of action partly in the pancreas: it increases the quantity of circulating insulin and, in addition, neutralizes the effect of insulin on the blood sugar level. Carbohydrate metabolism may proceed practically unchanged at a raised insulin level.

2 This rise in the level of insulin favours at least one of the actions of growth hormone on protein metabolism, namely, the shift of the equilibrium amino-acid ⇌ protein to the right. For the second effect, the inhibition of amino-acid breakdown in the liver, insulin is of less or perhaps of no importance.

3 The rise in the level of insulin impairs the utilization of fat as a source of energy in place of the protein which is lost This difficulty is combatted by a dual influence of growth hormone on fat metabolism peripheral mobilization and *increased* breakdown by the liver

4 This integration of the effects of growth hormone guarantees the fulfilment of caloric demands during its sparing action on proteins

REFERENCES

1 ANDERSON, E and LONG, J A, *Recent Progr Hormone Res*, 2, 217 (1948)
2 ASTWOOD, E B, personal communication
3 BENNETT, L L, GARCIA, J F and LI, C H, *Proc Soc exp Biol, N Y*, 68, 349 (1948)
4 BENNETT, L L, KREISS, R E and LI, C H, *Amer J Physiol*, 152, 210 (1948)
5 BENNETT, L L and LI, C H, *J clin Endocrin*, 6, 462 (1946)
6 BEST, C H, CAMPBELL, J, HAIST, R E and HAM, A W *J Physiol*, 101, 17 (1942)
7 BOERE, J M and GAARENSTROOM J H, *Versl gewone Vergad Akad Amst*, 52, 453 (1943)
8 BORNSTEIN, J, REID, E and YOUNG, F G, *Nature, Lond*, 168, 903 (1951)
9. CAMPBELL, J, DAVIDSON, I W F, SNAIR, W D and LEI, H P, *Endocrinology*, 46, 272 (1950)
10 COTES, P M, REID, E and YOUNG, F G, *Nature, Lond*, 164 209 (1949)
11 DE BODO, R C, KURTZ, M, ANCOWITZ, A and KIANG S P, *Proc Soc exp Biol N Y*, 74, 524 (1950)
12 DOHAN, F C and LUKENS, F D W, *Endocrinology*, 42, 244 (1948)
13 EVANS, H M, MEYER, K, SIMPSON, M E and REICHERT, F L., *Proc Soc exp Biol, N Y*, 29 857 (1932)
14 FRAENKEL-CONRAT, H, SIMPSON, M E and EVANS, H M, *Amer J Physiol*, 138, 439 (1943)
15 FRAENKEL-CONRAT, J, FRAENKEL-CONRAT, H and EVANS, H. M, *Amer J Physiol*, 137, 200 (1942)
16 FREUD, J, LEVIE, J A and KROON, D B, *J Endocrin*, 1, 57 (1939)
17 GAARENSTROOM, J A, *Proc Acad Sci Amst*, 49, 265 (1946)

18 GAARENSTROOM, J H, HUBLÉ, J and DE JONGH, S E, *Acta endocr, Copenhagen*, 2, 317 (1949)
19 GAARENSTROOM, J H, HUBLÉ, J and DE JONGH, S E, *J Endocrin*, 6, 71 (1949)
20 GAARENSTROOM, J H and DE JONGH, S E, *Acta brev neerl Physiol*, 14, 28 (1946)
21. GAARENSTROOM, J H and DE JONGH, S E, *Proc Acad Sci Amst*, 51, 166 (1948)
22 GAARENSTROOM, J H and KRET, A, *Proc Acad Sci Amst*, 48, 537 (1945)
23 GAEBLER, O H and ROBINSON, A R, *Endocrinology*, 30, 627 (1942)
24 HOBERMAN, H D, *Yale J Biol Med*, 22, 341 (1950)
25 HOUSSAY, B A, *Endocrinology*, 30, 884 (1942) and earlier papers
26 HOUSSAY, B A and ANDERSON, E, *Endocrinology*, 45, 629 (1949)
27 KOCHAKIAN, C D and STETTNER, C E, *Amer J Physiol*, 155, 262 (1948)
28 KURTZ, M, DE BODO, R C, KIANG, S P and ANCOWITZ, A, *Proc Soc exp Biol, N Y*, 76, 21 (1951)
29 LI, C H, SIMPSON, M E and EVANS, H M, *Endocrinology*, 44, 71 (1949)
30 LONG, C N H, *Diabetes*, 1, 3 (1952)
31 LUKENS, F D W and DOHAN, F C, *Endocrinology*, 30, 175 (1942)
32 LUKENS, F D W, DOHAN, F C and WOLCOTT, M W, *Endocrinology*, 32, 475 (1943)
33 MAASSEN, A P, *Proc Acad Sci Amst*, 54, 160 (1951)
34 MAASSEN, A P, *Arch int Pharmacodyn*, 88, 434 (1952)
35 MACKAY, E M, BARNES, R H and BEGMAN, H C, *Amer J Physiol*, 126, 155 (1939)
36 MARX, W, HERRING, V V and EVANS, H M, *Amer J Physiol*, 141, 88 (1944)
37 MILMAN, A E and DEMOOR, P, *Fed Proc*, 9, 90 (1950)
38 MILMAN, A E and RUSSELL, J A, *Fed Proc*, 8, 111 (1949)
39 MIRSKY, I A, *Amer J Physiol*, 124, 569 (1938)
40 MIRSKY, I. A, *Endocrinology*, 25, 52 (1939)
41 RICHARDSON, K C and YOUNG, F G, *J Physiol*, 91, 352 (1937)
42 RUSSELL, J A and CAPIELLO, M, *Endocrinology*, 44, 520 (1949)
43 SCHAEFFER, N K. and LEE, M, *J biol Chem*, 108, 355 (1935)
44 SZEGO, C M and WHITE, A, *Endocrinology*, 44, 150 (1949)
45 WEIL, R. and ROSS, S, *Endocrinology*, 45, 207 (1949)
46 VAN WIERINGEN, G, *Acta. brev neerl Physiol*, 14, 74 (1946)

47 VAN WIERINGEN, G , *Arch int Pharmacodyn* , 36, 450 (1948)
48 VAN WIERINGEN, G and DE JONGH, S E , *Proc Acad Sci Amst* , 52, 835 (1949)
49 WIJNANS, M and DE JONGH, S E , *Acta physiol pharm neerl* , 2, 74 (1951)
50 WOERNER, C A , *Anat Rec* , 71, 33 (1938)
51 YOUNG, F G , *Lancet*, ii, 372 (1937)
52 YOUNG, F G , *J Endocrin* , 1, 339 (1939)
53 YOUNG, F G , *Brit Med J* , ii, 897 (1941)
54 YOUNG, F G , *Biochem J* , 39, 515 (1945)
55 YOUNG, F G , *J clin Endocrin* , 10, 824 (1950)

DISCUSSION

YOUNG I entirely agree that the name 'diabetogenic hormone' is not a good one to use, since the production of diabetes is obviously not a physiological result of the secretion of the hormone

I should like to refer briefly to some work carried out in our laboratory and published in 1951 (BORNSTEIN, J , REID, E and YOUNG, F G , *Nature, Lond* , 168, 903 1951) We used alloxan diabetic, hypophysectomized adrenalectomized (ADHA) rats and determined the response of the blood sugar level to blood from other animals Briefly our findings were as follows

(i) Growth hormone had little direct effect on these ADHA animals However, if blood from such an animal treated with growth hormone was taken twenty-four hours later and injected into a second ADHA rat a rise in the blood sugar level occurred in the recipient animal One possible explanation of this effect is that the growth hormone stimulated the production of glucagon in the donor rat

(ii) Normal cats were treated with growth hormone for five or six days, so that a state of idiohypophyseal diabetes was produced, and blood was then taken from the pancreatico-duodenal vein and injected into ADHA rats This provoked hyperglycaemia However, if the blood was taken after only two days, a slight (perhaps not significant) hypoglycaemia was produced It seems possible that an extra secretion of insulin by the pancreas occurred first of all and that this was later counteracted by the secretion of an excess of glucagon

(iii) Blood was taken from the femoral vein of a cat rendered diabetic by treatment with growth hormone A fall in the blood sugar level (although perhaps not a significant one) occurred in the recipient ADHA rat This may indicate that there was some effective insulin in the peripheral blood although none could be detected in the pan-

creatico-duodenal blood Alternatively, it is possible that insulin was present in the pancreatico-duodenal blood, but that the same amount in the peripheral blood produced a greater effect because here the insulin antagonist was no longer effective for some unknown reason

These results are in keeping with the view that the hypoglycaemic-glycogenolytic factor is liberated from the pancreas—and perhaps also some insulin at the same time—under the influence of growth hormone. This idea will perhaps explain the results obtained by Anderson which gave no evidence of the liberation of insulin by the perfused rat pancreas when growth hormone was added to the perfusing fluid

DE JONGH I am in complete agreement with these views

RUSSELL. Some work carried out recently in our laboratory on the effects of growth hormone on the metabolism of nitrogen in eviscerated or in nephrectomized rats has in part confirmed some of the work described by Professor de Jongh We carried out three experiments

(i) Using eviscerated rats, we followed the change in the blood or plasma amino-nitrogen level with time For the first two hours there was very little change After that, the plasma amino-nitrogen level began to rise steadily and this increase could be partially inhibited by insulin In our hands, growth hormone alone had no effect on the release of amino-nitrogen into the plasma, but if given with insulin it produced a further inhibition Since the doses of insulin needed were rather large, the precise significance of this synergism is not clear

(ii) In another series of experiments, casein hydrolysate was given intravenously to eviscerated rats, beginning about two hours after the operation The plasma amino-nitrogen level, elevated by the infusion, fell to a stable level ½ to 1 hour after the injection and then began to rise on a course parallel to, but at a higher level than, that in the animals not given amino-acids If growth hormone was given to the animals before the injection of the hydrolysate the intermediate level to which the plasma amino-nitrogen content fell was distinctly lowered From this it appears that if the supply of amino-acids is sufficient, growth hormone may induce retention of nitrogen in the peripheral tissues in the absence of the liver and also in the absence of all but traces of insulin.

(iii) We studied the accumulation of urea in the blood of nephrectomized rats to obtain a measure of nitrogen catabolism Growth hormone alone had little effect on the rate of urea formation in the fasting animal. When casein hydrolysate was given intravenously however, the rate of urea formation, much increased over that seen in the fasting controls, was depressed markedly by the prior administration

indeed Glucose produced some stimulation but the rate was not normal unless insulin was added as well In the presence of insulin alone there was no measurable incorporation Therefore, in the tissue from the diabetic animal the effect is dependent upon the presence of both carbohydrate and insulin

YOUNG Professor Best said earlier on that extra insulin may not be necessary for the activity of growth hormone to be expressed but that this does not mean that extra insulin is not secreted under the influence of growth hormone under physiological conditions With this I am in complete agreement

NITROGEN RETENTION AND THE ACTION OF INSULIN

By

F D W LUKENS

The George S Cox Medical Research Institute, University of Pennsylvania, Philadelphia, Pennsylvania, U S A

INTRODUCTION

Since the action of insulin is unknown, except by its various physiological effects, 'The Influence on Nitrogen Retention' might more appropriately express the topic of this paper For purposes of this discussion, the term nitrogen retention should be defined The retention of nitrogen commonly refers to the nitrogen balance of the organism, which in turn measures the extent of protein anabolism or catabolism of the body as a whole The nitrogen balance is negative in starvation or stress, it is in fairly stable equilibrium in the adequately fed adult, and the balance becomes positive under the influence of growth hormone The role of insulin in nitrogen retention must be viewed against the background of these various conditions Furthermore, it is appropriate to recall some of the methods used to study the nitrogen balance of the body These methods include the usual metabolic measurements, in which dietary, urinary and faecal nitrogen are determined Other methods are body growth, measured as gain in weight or in length (*i e*, size) or both, gain or loss of weight under certain circumstances, changes in the composition of the carcass, especially in its protein or nitrogen content, changes in the rate of accumulation of blood nitrogen in nephrectomized animals, and, more recently, the retention of nitrogen has been followed by the incorporation of isotopically labelled amino-acids All of these methods for the study of nitrogen metabolism have been employed in experiments in which the important metabolic hormones have been removed or administered under different conditions

From this large amount of material I shall try to select the principal observations which pertain to the influence of insulin on nitrogen (protein) metabolism As the parts played by insulin and growth hormone are particularly considered, two things must be kept in mind first, the fact that the effect on nitrogen

both glutathione
was then added t
In slices taken fro
indeed Glucose
normal unless ins
alone there was n
from the diabetic
of both carbohyd

YOUNG Profess
be necessary for tl
that this does not
influence of growt
thus I am in compl

constant dose of cortical hormone (10) In whatever way the adrenal cortex affects nitrogen metabolism, we know that it can exert this influence in the absence of the pancreas (Houssay animal) and that it also exerts a significant anti-insulin action in rats (9) and in man (4) Engel *et al* have examined the relation between the growth and adrenocorticotrophic hormones in the production of glycosuria in the rat (7) Important as the adrenal cortex is, it can be cited here only as a variable to be kept in mind in discussing insulin and nitrogen retention

Growth hormone and the androgens, especially testosterone, exert an anabolic action on protein metabolism (11) In both cases the presence of insulin and of a suitable level of thyroid function is apparently required if the optimal increase in nitrogen retention is to occur The difference in the nature and duration of the nitrogen retention induced by testosterone and growth hormone is recognized, though not fully understood Unlike growth hormone, the androgens have no anti-insulin action After this rapid summary of the factors regulating protein metabolism, certain experiments on the behaviour of insulin and growth hormone may be examined

THE REMOVAL OF INSULIN

The increased excretion of nitrogen by the fasting depancreatized dog was established by Minkowski It remained for Houssay to show that this severe protein-catabolic response to the loss of insulin was due to the unrestricted activity of the anterior pituitary Soon after this, Dr Long and I found that the adrenal cortex was largely responsible for this pituitary effect The increased nitrogen excretion of the severely diabetic animal thus appeared as a secondary effect of insulin deprivation Since the actual nitrogen excretion was essentially the same in the normal and Houssay animals, one would conclude from these experiments that insulin did not primarily or directly affect protein metabolism. Some caution may be needed in drawing this conclusion because of the drastic nature of the experiments

THE ADMINISTRATION OF INSULIN

Studies on the administration of insulin to normal animals or man have led to contradictory reports (17, 20) Under various

retention is only one of the responses to these hormones which are also important in the metabolism of carbohydrate and fat, and second, other factors which affect protein metabolism.

STUDIES IN NORMAL ANIMALS

In the normal animal or man, nitrogen retention takes place in the presence of an intact system of regulatory hormones Studies of the nitrogen balance have increased the knowledge of the nutritional problems related to normal body growth and maintenance They also show the extent to which the body can adjust itself to unusual events As a result, the essential problems of quality, quantity and assimilation of the various foodstuffs must be remembered as this inquiry about the function of hormones progresses In an excellent review on *Carbohydrate and Fat as Factors in Protein Utilization and Metabolism* (16), Munro summarizes the roles of the three major foodstuffs and of calories, exercise and other factors, including hormones, in the metabolism of protein. However, in order to understand the part played by any one hormone a more detailed 'endocrine analysis' is required

The hormones which are principally concerned in the regulation of protein metabolism are the thyroid hormone, growth hormone, insulin, testosterone and the adrenal cortical hormones of the cortisone type The thyroid hormone appears to act on protein metabolism indirectly by its effect on the caloric needs However, as Talbott and Sobel have pointed out (22), clinical experience with cretins and dwarfs, as well as much experimental work, indicates that the thyroid hormone is an 'essential facilitator' of growth, for which proper thyroid function is required This probably means that a suitable level of thyroid function is necessary, not that the thyroid hormone has any specific effect on protein metabolism. With this passing comment, the thyroid hormone will be dismissed

The principal catabolic hormones are those of the pituitary-adrenal cortical axis, the most potent effector hormones being the 11-oxycorticosteroids such as cortisone Protein catabolism requires the presence, but not the increased secretion, of these hormones Ingle showed that nitrogen loss after injury did not occur in adrenalectomized rats maintained on salt solution but occurred to the usual degree in adrenalectomized rats kept on a

and insulin. Thus, Ingle with rats (9) and Conn with man (4) have found that insulin in large amounts did not counteract the negative nitrogen balance produced by ACTH or cortisone In diabetics, however, the increased excretion of nitrogen induced by ACTH or cortisone was readily controlled by increasing the dose of insulin (3) It is impossible to tell from these results whether or not insulin directly influences nitrogen excretion since it may have failed to act because of the insulin-resistant state For the present, these and many other studies on the adrenals must be set aside in order to look at the relation between insulin and growth hormone

THE REMOVAL OF GROWTH HORMONE (HYPOPHYSECTOMY)

Although hypophysectomy removes more than the growth hormone, the effectiveness of purified growth hormone (essentially free of thyrotrophic and adrenocorticotrophic activity) in restoring the growth of hypophysectomized animals indicates its primary importance in protein anabolism Two observations on the untreated hypophysectomized animal are particularly relevant

(i) Rats hypophysectomized prior to six days of age continue to grow until about forty days of age (23) This apparently means that the tissues have an inherent capacity for growth which in [illegible]

observations prepare one for the possibility that conditions might exist in which some growth also occurs in the absence of insulin

(ii) In adult animals the impaired growth after hypophysectomy is observed in the presence of ample insulin and of insulin sensitivity However, the emaciation of the hypophysectomized animal, which is due to the reduced intake of food, can be prevented by forced feeding (19) Even forced feeding conserves only twenty to thirty per cent of the nitrogen retained by pair-fed normal rats

The loss of body protein in hypophysectomized rats (12, 19) and the gain in protein under the influence of growth hormone (13) have been demonstrated by carcass analysis Under some conditions, these measurements are more sensitive indicators of protein anabolism than changes in body weight or size and they

circumstances there may be an increase, no change, or a decrease in nitrogen excretion In addition to different experimental conditions, the counter-regulatory effects of other glands may be responsible for these discrepancies In response to the administration of insulin, the secretion of epinephrine is increased (5) and the insulin *content of the pancreas is greatly diminished* (2) Clouded with such difficulties in interpretation, the effects of insulin in normal animals add little precise information on the part played by any single hormone

When insulin is administered to the depancreatized animal, the excess protein catabolism is prevented along with the effects observed on carbohydrate and fat metabolism As a rule, the nitrogen balance is restored to normal but does not go beyond this point, that is, no significant protein anabolism occurs This clearly demonstrates that insulin has an important function in promoting nitrogen retention, provided one employs appropriate experimental conditions

At this point the attempts to measure a nitrogen-sparing function of glucose in the absence of insulin may be mentioned In 1937, Barker and Sweet, and Mirsky, Heiman and Swadesh, observed that when depancreatized nephrectomized animals were given large amounts of glucose, there was a significant retardation in the rate of accumulation of non-protein nitrogen in the blood (1, 15) Munro accepts these results as showing that 'the islets of Langerhans are not essential in the protein-sparing

is apparently required Secondly, in these brief reports there is no mention of the administration of a control solution of corresponding osmolar concentration such as urea, sucrose or salt Thirdly, some of our results (obtained since Munro's review appeared) support the conclusion that the presence of insulin is essential for protein sparing, at least in the sense of bringing about protein anabolism

The effects of insulin described thus far have occurred in the presence of the pituitary gland Recent advances in the study of the adrenocorticotrophic and growth hormones have led to a number of investigations on the relation between these agents

adjusted every four to six days so that a metabolic period was obtained at each level of insulin dosage (14) Under these conditions, it was found necessary to increase the original maintenance dose of insulin three- to fivefold before the normal degree or nitrogen retention occurred Just as the studies made on a constant insulin regimen show that the normal animal secretes insulin in response to growth hormone, so the results with increasing [illegible]

is diabetogenic

HYPOPHYSECTOMIZED DEPANCREATIZED (HOUSSAY) CATS RECEIVING NO INSULIN

Previous experiments have not included the administration of purified growth hormone to animals in the absence of insulin Since the usual survival time of untreated depancreatized cats is three days, suitable metabolic periods without insulin would be impossible For this reason Houssay cats were prepared and were stabilized on a constant diet without insulin after recovery from the operations The diet was 100 g instead of the usual 150 g of meat daily, but normal cats can show marked nitrogen retention when given growth hormone on the 100 g diet Table I shows that in the complete absence of insulin growth hormone, even at the larger dosage, caused *no retention of nitrogen* This is based on the comparison between a control period and a period on growth hormone In Table II the results of these experiments with doubly operated animals have been presented in another way The metabolic periods which were consecutive, and the doses of hormone used are given The observed urinary nitrogen excretion is tabulated and the balance is calculated as the difference between nitrogen excreted and *dietary* nitrogen Even when only the urinary and dietary nitrogen are considered, these animals are seen to be in doubtful equilibrium When one recalls the faulty absorption of food by the depancreatized animal even when pancreatic extract is added to the [illegible]

of observation Certainly, growth hormone caused no nitrogen

most instances There was some difference in body weight which may be related to this but which cannot be the only reason for the variable insulin requirement In any case, when the insulin need of an animal has been determined and when administration [illegible]

is significant In this connection, three depancreatized cats (not tabulated) had their insulin increased by fifty per cent at times when they received no growth hormone These increments of insulin caused no nitrogen retention Larger increments could not be tested because of the danger of hypoglycaemia

Third, when the amount of insulin given is kept constant, 10 mg of growth hormone causes significantly greater nitrogen retention than 3 mg of hormone In fact, the amount of the *nitrogen retained under the influence of the dose of 10 mg of* growth hormone in the depancreatized cats equalled the average amount of nitrogen retained by the dose of 3 mg in the normal animals This suggests that growth hormone, rather than insulin, exerts the dominant influence on nitrogen storage, even though an ample supply of insulin is necessary for an optimal response

DEPANCREATIZED ANIMALS GIVEN INCREASING AMOUNTS OF INSULIN DURING THE ADMINISTRATION OF GROWTH HORMONE

Gaebler and Robinson performed such an experiment in dogs, with the result that almost normal nitrogen retention was observed with their crude growth hormone, which had caused nitrogen loss when the amount of insulin given was constant (8) In this study there was a control period followed by administration of a single large dose of pituitary powder *On this day and for one to* two days thereafter the amount of insulin given was increased two- to threefold Nitrogen retention was usually maximal on the second day and lasted for about four days This duration of action of growth hormone has been seen in another form by De Bodo, who noted progressively increasing insulin resistance when small doses of purified growth hormone were given daily for two to three weeks (6) In our experiments small daily doses of growth hormone were used and the size of the insulin dose

hormone had a greater contra-insulin action on fat formation than on other pathways of carbohydrate metabolism The same result might be due to a difference in the action of insulin on these metabolic pathways since the result depends upon the equilibrium between these hormones Although growth hormone antagonizes

are needed for this effect to present itself and these conditions occur when the hormone is given to normal animals It is probable that in normal growth the changes are less drastic than those produced by the administration of growth hormone in the laboratory, but all who have treated diabetic children know that growth poses metabolic problems.

In conclusion, the studies on the effect of growth hormone and of insulin on the retention of nitrogen show that these two agents are part of the total hormonal regulation of protein metabolism The results to date support the following concepts

1 Insulin is essential to the protein anabolic action of growth hormone

2 An increased secretion of insulin apparently occurs in response to the administration of growth hormone to normal animals This is inferred from the impaired nitrogen retention of depancreatized animals given growth hormone, from the restoration of normal nitrogen retention in such animals by increased amounts of insulin, and from other evidence

3. This increased demand for insulin may well exceed the functional reserve of the pancreatic islets and thus account for the

studies in Houssay animals)

5. Although insulin is regarded, for the present, as an essential

retention in the absence of insulin when the results are viewed in this manner.

TABLE II

Nitrogen balances of Houssay cats

All animals were fed daily 100 g of meat which contained 3 3 g of nitrogen by analysis

Cats	Period (days)	Dose of growth *hormone* (mg /day)	Average urinary nitrogen excretion *observed* (g /day)	*balance** (g /day)	Weight (kg)
	4	0	2 9	+0 4	1 9
M–21	4	3	2 9	+0 4	
	4	0	2 8	+0 5	1 9
M–23	4	0	3 8	—0 5	2 5
	4	10	3 7	—0 4	2 5
M–26	5	0	3 3	0 0	2 6
	4	10	3 6	—0 3	2 5

*Faecal nitrogen was not determined—see text

In the depancreatized animals of Table I and in the Houssay [illegible] In the

stant insulin dosage, which had some nitrogen retention it seems proper to conclude from this that insulin is an essential [illegible]

played by the wastage of carbohydrate in such experiments

CONCLUSIONS

In viewing the studies on growth hormone, insulin and nitrogen retention as a whole, one seeming discrepancy merits comment Growth hormone produces marked insulin resistance (6), but at the same time appears to evoke an increased secretion of insulin (8, 14) In the normal animal, this increased secretion of insulin prevents glycosuria and facilitates the storage of nitrogen At the same time a diminished deposition or actual loss of body fat takes place and ketogenesis may occur It appears that a special equilibrium between these hormones is needed for optimal protein anabolism. The findings might be explained if growth

22 TALBOTT, N B and SOBEL, E H, *Recent Progr Hormone Res*, 1, 355 (1947)
23 WALKER, D G, SIMPSON, M E, ASLING, C W and EVANS, H M, *Anat Rec*, 106, 539 (1950)
24 YOUNG, F G, *Biochem J*, 39, 515 (1945)

DISCUSSION

CONN If growth hormone preparations are given to man in small quantities they neither induce diabetes nor stimulate retention of

[illegible] in man of

of Raben

paration is

[illegible]

there is probably still some residual functioning of both the pituitary and the pancreas The dose administered was 1 mg per kg intramuscularly every twelve hours

The results indicate that this preparation induced a clear-cut positive nitrogen balance, amounting to a retention of 1 g of nitrogen daily The addition of a daily dose of 10 units of protamine zinc insulin did not increase the degree of nitrogen retention In confirmation of Professor Best's experience, we found however that insulin *alone*, if given at the limit of the patient s tolerance (in this case 15 units of protamine zinc insulin daily) induced a retention of 1 5 g of nitrogen daily Therefore it is possible that, in our study, the growth hormone had stimulated residual pancreatic function and that the resultant

[illegible]

did not occur

It would appear that we now have a preparation of growth hormone which is effectively growth-promoting in man and which is still not diabetogenic. However, much more work will be required before the results obtained in man to date can be reconciled with the experiments on animals

synergist in nitrogen retention, the level of growth hormone seems to exert the dominant effect in the control of nitrogen metabolism and seems to be independent of minor variations in the supply of insulin

REFERENCES

1 BARKER, S B and SWEET, J E, *Science*, 86, 270 (1937)
2 BEST, C H and HAIST, R E, *J Physiol*, 100, 142 (1941)
3 BROWN, E M, Jr, LUKENS, F D W, ELKINTON, J R. and DEMOOR, P, *J clin Endocrin*, 11, 1363 (1950)
4 CONN, J W, LOUIS, L H and JOHNSTON, M W, *J Lab clin Med*, 34, 255 (1949)
5 CORI, C F, *Physiol Rev*, 11, 143 (1931)
6 DE BODO, R C, KURTZ, M, ANCOWITZ, A and KIANG, S P, *Amer J Physiol*, 163, 310 (1950)
7 ENGEL, F L, VIAU, A, COGGINS, W and LYNN, W S, Jr, *Endocrinology*, 50, 100 (1952)
8 GAEBLER, O H and ROBINSON, A R, *Endocrinology*, 30, 627 (1942)
9 INGLE, D J, SHEPPARD, R., EVANS, J H and KUIZENGA, M H, *Endocrinology*, 37, 341 (1945)
10 INGLE, D J, WARD, E O and KUIZENGA, M H, *Amer J Physiol*, 149, 510 (1947)
11 KOCHAKIAN, C D, *Amer J Physiol*, 160, 66 (1950)
12 LEE, M and AYRES, G B, *Endocrinology*, 20, 489 (1936)
13 LI, C H, SIMPSON, M E and EVANS, H M, *Endocrinology*, 44, 71 (1949)
14 MILMAN, A E, DEMOOR, P and LUKENS, F D M, *Amer J Physiol*, 166 354 (1951)
15 MIRSKY, I A, HEIMAN, J D and SWADESH, S, *Amer J Physiol*, 120, 681 (1937)
16 MUNRO, H N, *Physiol Rev*, 31, 449 (1951)
17 PETERS, J P and VAN SLYKE, D D, *Quantitative Clinical Chemistry*, vol I, 2nd ed., Baltimore The Williams & Wilkins Co (1946)
18 RUSSELL, J A, *Amer J Physiol*, 140, 98 (1943)
19 SAMUELS, L T, REINECKE, R W and BAUMAN, K L, *Endocrinology*, 33, 87 (1943)
20 SOSKIN, S and LEVINE, R., *Carbohydrate Metabolism*, p 174, Chicago, Ill University of Chicago Press (1946)
21 STADIE W C, HAUGAARD, N, HILLS, A G and MARSH, J B, *Amer J med Sci*, 218, 275 (1949)

[illegible]

the Raben and Westermeyer preparation is relatively more favourable to its absorption by slow continuous infusion In other words, perhaps it favours growth rather than diabetogenesis Experiments with isolated tissues may also be affected by the variations in the physical state of the growth hormone in different preparations

GAARENSTROOM Experiments carried out by Professor de Jongh and myself show that hypophysectomized alloxan diabetic rats grow at the normal rate when treated with growth hormone This suggests that the presence of an intact pancreas is not necessary for growth to occur

LUKENS I believe that alloxan diabetic rats will also grow if given insulin

GAARENSTROOM Is the growth the same as in normal animals?

LUKENS I do not know what would happen to normal rats treated with insulin In alloxan diabetes there is a variable deficiency of insulin More information on the condition of the diabetes is needed

GAARENSTROOM If the animals are made diabetic with alloxan before hypophysectomy, two to three grams of glucose is excreted per day

LUKENS This is a moderate diabetes One would therefore expect some nitrogen to be retained

LONG The relative effects of ACTH and growth hormone are as ten to one in rats This can be compared with the results of Dr Camp-

[illegible]

not very likely that these large amounts of insulin were being secreted before the onset of the diabetes Also Langfeldt found over thirty

[illegible]

eventually died

YOUNG Reid has examined the preparation of Raben and Westermeyer and found it to be diabetogenic in cats, although the activity was only about one-third of that of other preparations of growth hormone The growth-promoting activity was also reduced to about the same extent (REID, E , *Ciba Foundation Colloquium on Hormonal Fac-*

retention of nitrogen in your experiments

LUKENS De Bodo tested the Raben and Westermeyer preparation on a hypophysectomized dog and found that it had one-third of the normal activity when assayed by its effect in reducing the sensitivity to insulin

CONN Ability to induce retention of nitrogen would have been a more satisfactory method of assay because the material is reputed to have low diabetogenic activity but high activity in stimulating nitrogen retention

LUKENS De Bodo thinks that it is more important to examine the

is have

tuitary

CONN No nitrogen retention occurs when the older preparations of growth hormone are given in cases of Simmonds' disease, but the effect of Raben's material on such cases has not yet been examined

LAWRENCE What was the intensity of the boy's diabetes in the ten-day period when he was without insulin and his blood sugar level was about 100 mg per cent?

CONN One would not expect to find intense diabetes in the hypopituitary diabetic Such individuals are excessively sensitive to insulin In the case reported above the blood sugar levels during fasting were in the range 100–110 mg per cent (The normal level is 70–90 mg per cent) During glucose tolerance tests, however, plateaus occurred on the curves above 400 mg per cent

LUKENS The effects observed seem to be markedly dependent on the properties, and particularly the solubility, of the preparation of growth hormone used

LONG There is no evidence that large doses of growth hormone have any effect on normal humans—not even on their carbohydrate metabolism—yet small amounts of the same preparation *induce* diabetes in the cat or dog

WILHELMI There is one case of a girl in which treatment with

PROBLEMS CONCERNING THE ABSORPTION OF INSULIN FROM HUMAN SUBCUTANEOUS TISSUE

By

H C HAGEDORN

Niels Steensens Hospital, Gentofte, Denmark

VASCULAR COMPLICATIONS IN DIABETES

The main problem in the treatment of diabetes today is the vascular complications They were seen but rarely in the pre-ins [illegible]
de [illegible]

expected to reduce the frequency of a certain complication if this complication is a consequence of the diabetic condition

If retinitis is a consequence of a vascular disease which is also [illegible]

order, it should be possible by a more rigorous compensation of this disorder to reduce the frequency of occurrence of the complication or cure it when it is already present Whether the one or the other of these alternatives is the real one, is difficult to decide on the basis of clinical experience, opinions differ on the question, although apparently the latter view is becoming more predominant

Some help has been derived from experimental science The work of Lukens and Dohan, who found intercapillary glomerulosclerosis in a dog with experimental pituitary diabetes (8), as well as the experiments of Foglia, Mancini and Cardeza on partially pancreatectomized animals (4) and the statement by Lawrence on the occurrence of blood vessel lesions in haemo- [illegible]

Niels Steensens Hospital by Dr J E Poulsen further supports this view a diabetic with retinopathy developed Simmonds' disease, with remarkable improvement in the diabetes and complete disappearance of the retinopathy

LUKENS The puppy is similar to the rat with regard to the hyperplasia produced

YOUNG I should like to reiterate the remarks made by Dr Lukens earlier in the symposium about the overall importance of the enzymic pattern within the tissues This is perhaps more important, with regard to age for example, than is at present realized

by glycosuria in about 78 per cent of the animals (66 7 per cent after ligation 86 7 per cent after establishment of the fistula) (3) He carried out 153 experiments and observed glycosuria ½–1½ hours after the operation which increased in severity up to the sixth hour The animals lived for some weeks or months with glycosuria—even during starvation or on a carbohydrate restricted diet Intravenous infusion of lymph serum into dogs with glyco suria was followed by a decrease in the output of glucose in the urine and he concluded that the chyle contains a substance [illegible]

then insulin is lost But with ligation this can hardly be so and the hypothesis that damage to the pancreas occurs may be the most likely Biedl also observed necrosis in the pancreas in a few of the animals It is remarkable that these investigations have not been followed up

But even if we assume that some insulin is discharged through the thoracic duct in dogs we should not conclude that the same takes place in man Naunyn in his book *Diabetes* mentions Biedl s work and quotes a personal communication from Gerhardt that Biedl s work could not be confirmed (nothing however has been mentioned in the literature) (9)

A single observation of the insulin content of chyle in man has been made in our laboratory by Dr Marie Weitze A portion of the pleural exudate from a patient with traumatic lesion of the thoracic duct was extracted with acid alcohol and purified The amount of insulin in the lymph was found to be 0 04 unit/ml (The determination was made on mice by the convulsion method) Some time after the removal of the chyle a sample of blood was taken (three hours after breakfast) The serum was treated in almost the same way as the lymph The insulin content of the serum was found to be 0 003 unit/ml by the method of Anderson Lindner and Sutton (1) It must be borne in mind that most sources of error would tend to give results which were too low With all necessary reservations this experiment therefore seems to indicate that the insulin concentration in the chyle is at least ten times as high as that in the blood But it does not seem pos-

It therefore appears, as a practical result that an all-out effort *for a full compensation of the* diabetic metabolic disturbance should be made in such cases where the circumstances, especially the age of the patient, make it probable that such complications *might be expected* *Up to the* present, the best indication that such compensation has been reached is a blood-sugar level within normal limits

UNKNOWN FACTORS CONTROLLING AND COUNTERACTING THE SECRETION OF INSULIN

The use of insulin in the therapy of diabetes depends, *inter alia* on the regulating forces of the organism When we inject insulin in a dose larger than that necessary to preserve normal metabolism, the organism puts into play a number of counteracting forces The onset of what we call a hypoglycaemic attack means that those forces have been overcome Yet in fact we do not know what undesirable effects we produce by bringing those forces into play, even if the patient does not show the well known signs of a hypoglycaemic attack This provides a further argument in favour of close control of the insulin dosage

A daily intake of 500 grams of carbohydrate and 3000 Calories —which is not excessive for a healthy subject—cannot be taken by a severe diabetic if full compensation of the diabetic symptoms [illegible] we are unable to imitate But before going further into this I wish to bring up for discussion another problem which may or may not be concerned with the limitations of insulin therapy

POSSIBLE ABSORPTION OF INSULIN VIA THE CHYLE

It is generally supposed that insulin is secreted by the pancreas into the portal vein If this is correct the insulin concentration in *the capillaries of the liver may be* much higher than in other parts of the body Whether some insulin also leaves the pancreas via the thoracic duct is not quite clear Biedl claimed in 1898 to have shown that ligation of the thoracic duct or the removal of the chyle by means of a fistula of the duct in the dog is followed



FIG. 2
Radiograph showing depot in the subcutaneous tissue

sible at present to adopt a therapy in which insulin is absorbed

But with subcutaneous injection, the fall does not occur until after thirty minutes (Fig 1) The delay following subcutaneous

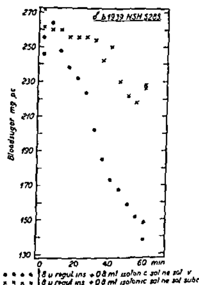


Fig. 1

Blood sugar values after intravenous or subcutaneous injection of insulin

The reabsorption of mixing it with hyal-

If a radio-opaque substance is injected into the subcutaneous

with the lymph vessels How much injected insulin enters the vessels and how much passes directly into the blood vessels is not yet known

At this point I should like to mention an observation by Peck. He found that acid (clear) solutions of insulin plus protamine,

histone or globin had a more variable, more erratic and more unpredictable effect than the comparable mixtures of the neutral series, that is, those in which the insulin was present in suspension (10) We have had similar experience, which seems to be common to all clear, slowly absorbed preparations of insulin The explanation of this phenomenon may be that the clear solu- [illegible]

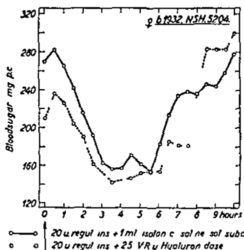


FIG 3
Blood sugar values after subcutaneous injection of insul n w th and withe it hyaluron dase

FACTORS AFFECTING THE ABSORPTION OF PROTAMINE INSULIN

My remaining remarks concern the absorption of the protamine insulin compounds The slow absorption of these compounds is supposed to be caused by their insolubility in blood plasma and tissue fluids

It has been shown that blood serum contains a protamine splitting enzyme (5) Izzo also studied the dissolving of various preparations of insulin in serum and found that globin insulin is *not* split by serum enzymes (6) Bang has demonstrated the presence of the same or a similar enzyme in the vesicular fluid

lysin The protamine-splitting enzyme in blood or tissue extracts is, like fibrinolysin, activated by streptokinase It is therefore most likely that the protamine-splitting factor is identical with fibrinolysin Poulsen has also found that zinc is an inhibitor of the enzyme and that the addition of streptokinase to protamine insulin abolishes the effect of zinc in delaying the onset of hypoglycaemia (Fig 6) In addition to zinc, glycerol and phenol have a slightly inhibitory effect on the enzyme

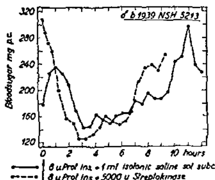


Fig 6

Blood s gar values after subcutaneous injection of insul n w th and w tho it streptok nase

It is well known that another enzyme system plays an important role in the absorption of insulin, namely, the 'spreading factor' This is also inhibited by zinc Hyaluronidase, when added to clear insulin before subcutaneous injection, does not promote the fall in the blood sugar concentration Hyaluronidase added

be achieved, however, by the use of mixtures Most patients will need a comparatively slowly working product during the night, but relatively more per hour after meals In some cases this has been done by giving a combination of soluble insulin and crystals of protamine insulin (NPH) in the morning The use of the crystalline product is especially advisable for mixtures, because the amount of insulin adsorbed to the crystals is much

from a bulla produced by an *emplastrum canthandis c euphorbio*(2) Zinc was found to be an inhibitor of the enzyme

We have been able to extract a similar or identical enzyme from human subcutaneous tissue with isotonic saline (Fig 4). Using

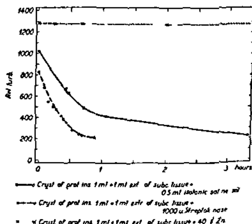


FIG 4

Effect of the enzyme from subcutaneous tissue on protamine insulin

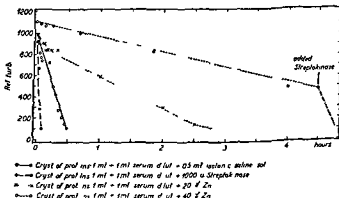


FIG 5

Effect of the enzyme from serum on protamine insulin.

Cohn's method of fractionation, Poulsen has demonstrated that the protamine-splitting enzyme in blood is found exclusively in the thrombin fraction (Fig 5) (11) This fraction contains fibrino-

less than to the same amount of an amorphous product (Fig 8) The absorption of protamine insulin can be delayed by inhibitors of the protamine-splitting enzyme If an inhibitor for the spreading factor is present too, the absorption of unmodified insulin may also be delayed

REFERENCES

1 ANDERSON, E, LINDNER, E and SUTTON, V, *Amer J Physiol* 149, 350 (1947)
2 BANG, H O, *Acta Pharm tox*, *Kbh*, 2 79, 89 (1946)
3 BIEDL, A, *Zbl Physiol*, 12, 624 (1898)
4 FOGLIA, V G, MANCINI, R E and CARDEZA, A C *C R Soc Biol*, *Paris*, 142, 1572 (1948)
5 HAGEDORN, H C, *Skand Arch Physiol*, 80, 156 (1938)
6 IZZO, J L, *Proc Amer Diabetes Ass*, 9, 225 (1949)
7 LAWRENCE, R D, *Brit med J*, II, 107 (1950)
8 LUKENS, F D W and DOHAN, F C, *Arch Path* (*Lab Med*), 41, 19 (1946)
9 NAUNYN, B, *Der Diabetes Mellitus*, 2nd ed, Vienna Hölder (1906)
10 PECK, F B, *Proc Amer Diabetes Ass*, 6 273 (1946)
11 POULSEN, J E, unpublished experiments

DISCUSSION

YOUNG It is remarkable that so little attention has been paid to Biedl's work Are you intending to do any further experiments to follow it up, Dr Hagedorn?

HAGEDORN No, not at present

LAWRENCE What is the usual route of absorption of subcutaneously injected insulin? I have always been under the impression that it occurs via the lymphatics but I have never been quite sure

HAGEDORN Some absorption certainly does occur through the lymphatics, but it is possible that some also occurs via the capillaries In the latter case, insulin may be split in the tissues into smaller units before being absorbed

LAWRENCE I agree with you that the use of mixtures of different preparations of insulin is a much more satisfactory procedure I find that, except in mild cases, no one form alone is yet available which will have both a strong initial action and yet last for twenty-four hours, unless such large doses are given that hypoglycaemia occurs

HAGEDORN If a mixture of crystalline protamine insulin and soluble

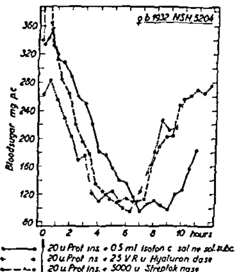


Fig 7

Blood sugar val es after subcutaneous inject on of insul n w th streptok nase or hyaluronidase

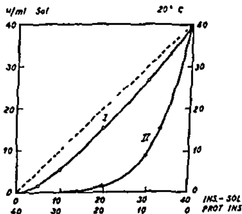


Fig 8

Curves showing adsorpt on of insul n to crystall ne protamine insul n and amorphous protamine z nc insul n at 20°C

THE INCIDENCE OF DIABETES AND HEREDITY

By

PER HANSSEN

Stavanger, Noru ay

STATISTICS

In the foreword to his book *The Treatment of Diabetes Mellitus*, Joslin refers to one of the main points facing future diabetic research in the following way 'One million diabetics in this country make diabetes a social problem which the laity, the medical profession and the Government should face' (14)

In order to solve this problem in the best possible way we need, among other things, full information about the frequency of the disease, its dependency on age, sex, mode of life etc We also need supplementary information about causal factors in order to prevent the disease

In the book [illegible] referred to [illegible]

has h therto been [illegible]

mortality compared with Massachusetts and Rhode Island, for example The results were summarized as follows 'We believe the incidence of diabetes is highest where (i) the average age is the oldest, (ii) women predominate, (iii) obesity is most frequent, (iv) the proportion of Jews is greatest, (v) medical supervision is closest, and (vi) deaths are most accurately reported' (13) Joslin finds, therefore, that the investigations in Arizona support the thesis that 'diabetes is universal'

The age at which diabetes becomes apparent is remarkably constant in the different sets of statistics We can therefore not be far wrong in saying that most diabetics become so between the ages of forty and sixty In a given community, about 25 per cent

insulin is injected, there is a rapid effect from the soluble insulin and a prolonged effect from the crystalline protamine insulin This is possible because the soluble insulin is adsorbed far less to the crystals than it would be to an amorphous product But we are not so ambitious as to try to control *severe* diabetics with only one daily injection.

LONG How does the insulin content of the lymph compare with that of the blood in the pancreatic vein?

HAGEDORN We are not at all sure, as the few values which we have obtained are about ten times higher than Bornstein's However, there seem to be differences in technique

[illegible]

system at low blood sugar levels

HAGEDORN Something probably happens in the local tissues By the use of radioactive zinc, Root has found that the zinc remains in the tissues even when the insulin is exerting its full effect and is presumably dissolved in the blood When a patient injects insulin into a site where relatively considerable amounts of zinc are already present in the tissue, quite unpredictable rates of absorption may be expected

LONG Am I correct in believing that the action of the enzyme which splits protamine insulin is to destroy the protamine rather than the link between protamine and insulin?

HAGEDORN If a suspension of protamine insulin at pH 7 is treated with one of the splitting enzymes, the insulin goes into solution, but precipitates again if more protamine is added

which to build up a campaign against such a national scourge as diabetes

PUBLIC HEALTH SERVICES AND DIABETES

During the last twenty to thirty years, public health measures have brought the infectious diseases under control and reduced infant and child mortality to a minimum Public health services have therefore in recent years been able to pay greater attention to the study of the chronic diseases, geriatrics-gerontology and mental hygiene

The place taken by diabetes among the chronic diseases was such that in 1900 in the United States of America it was No 27 on the list of causes of death In 1948 it was No 8, being surpassed only by tuberculosis, pneumonia, nephritis, violent death, apoplexy, cancer and heart disease as causes of death

It has seemed natural to public health services to combat diabetes by that method which has already shown itself to be so successful, that is the epidemiological method, which can be defined as follows 'The science which concerns itself with the natural history of disease as it is expressed in groups of persons related by some common factor as age, sex race, location or occupation, as distinct from the development of disease in individuals' (20)

It is of essential importance to the public health point of view that many recent investigations have confirmed Naunyn's old teaching that the earlier diabetes is diagnosed and the more carefully it is treated, the better the prognosis

In 1947, the United States Public Health Service examined the sugar in the blood and urine of 3516 of the 4983 inhabitants of Oxford, Massachusetts (25) Two score of them were already known to be diabetics and thirty more of them were found to be so by this examination Thus the diabetic morbidity of the whole of this town could be taken to be 17 per mille, varying from nil under the age of fifteen to 51 per mille between the ages of *sixty-five and seventy-four These figures were considerably* higher than those which most observers had hitherto found, that

of its diabetics will be under fifty, 25 per cent between fifty and sixty, and about 50 per cent will be sixty or more

The following questions are of medico-social importance to what extent has the number of diabetics risen in recent years? and, how will diabetes develop in the future? It is generally

that the number of diabetics in the United States of America rises every year by about 50,000 An investigation in Bergen, which has a population of 100,000, showed that there was between a twofold and a threefold increase in the diabetic morbidity between 1925 and 1941 (7)

were markedly overweight, 25 per cent were moderately overweight, and only 15 per cent had a normal or subnormal weight before the onset of diabetes (1) It has also been a point of general agreement that loss of weight by a fat diabetic has a favourable effect on his disease

Naunyn was the first to emphasize the vital importance of

subject although different observers do not yet agree what are the special conditions under which the disease is transmitted by heredity

In 1940, Wilder commented on the investigations by White and Pincus in their article *Heredity in Diabetes* (23), as follows 'I am not sufficiently trained, either in statistics or eugenics, to be able to pass final judgment on this evidence, but it impresses me as being of more importance than anything else that we know about diabetes If it is not adequate, the subject cries aloud for

diabetics' (24)

was required before the carbohydrate metabolism could be regarded as abnormal Yet 33 per mille of the total number of persons examined showed too high a blood sugar Indeed, the figure for coloured women over fifty was as high as 79 per mille [illegible]

not yet known

The mass examinations already undertaken have raised two problems for which a solution must be found before these investigations can be proceeded with on a still greater scale The first of these problems is concerned with the mode of execution of the Screening Survey Is the determination of sugar in the urine to be the only test? At what time in relation to meals is this test to be undertaken? Which method is to be employed? Should a blood sugar determination supplement the determination of the sugar in the urine? If so, which test is to be used?

The next problem concerns the character of the examination of those persons in whom the Screening Survey has shown a likelihood or a certainty of changes in the carbohydrate metabol- [illegible]

test?

Best and his co-workers, and Harting and Glenn, have discussed this problem very carefully (12, 15) but it is still difficult to make sure that any one particular procedure is the best There can be no doubt that a series of supplementary investigations is necessary with regard to the actual procedure Even the method of carrying out the glucose tolerance test, in use for many years, is still much under debate, particularly in the United States of America, although there is unanimity over the technique in England and the Scandinavian countries

OBESITY AND DIABETES

[illegible]

is, 3–7 per mille, and they needed to be confirmed as soon as possible Certainly Blotner had found a very high morbidity by examining recruits in Boston (2), but one could not forthwith assume that there was a correspondingly high diabetic morbidity in the whole of this community

During the last few years the actual diabetic morbidity in the [illegible]

manifest diabetes, have been examined The United States Public Health Service has participated in these investigations, having been inspired and to some extent led by the American Diabetes Association and having co-operated with local medical societies and committees of laymen

The American Diabetes Association sponsors and promotes a continuing Diabetes Defection Drive, annually renewed by Diabetes Week, and through this action 'the Association hopes to educate the general public to the necessity of early discovery and control of the disease A successful programme of this kind, coupled with constant advances in therapeutic techniques, will eventually remove diabetes from the list of the ten major causes of death by disease in this country'

Hitherto, the results of these investigations have been published only to a limited extent Best and his co-workers have examined the blood and urine of 81 per cent of the population of Newmarket, Ontario (population 4800), excluding children under school age (15) These examinations revealed fifty-four cases of diabetes in twenty-one of which the disease had hitherto been unsuspected Thus there was a morbidity of 12 per mille In Jacksonville, Florida, Ford and his co-workers investigated the [illegible] persons with blood relations [illegible] ree new [illegible] ve times

as great as that which had been found for the whole of the population In Georgia more than 300,000 persons presenting themselves for mass surveys for tuberculosis and syphilis in recent years have undergone blood sugar determinations by the anthrone method (18) It was stated that a relatively high blood sugar concentration

[illegible]

[illegible]

tween the two forms of diabetes be proven

It is possible that the demonstration by Lawrence and Bornstein of differences in the insulin content of the blood in old and young diabetics (3) may be helpful in pursuing these genetic investigations further, for one could, for example, investigate the hereditary conditions of diabetics in whose blood there is no demonstrable insulin and compare the findings with those from diabetics in whom insulin is shown to be present in the blood

This international symposium on 'Experimental Diabetes and its Relation to the Clinical Disease' must undoubtedly lead to the treatment of clinical diabetes being made more effective and to greater importance being attached to the prophylaxis of clinical diabetes But if we are to exploit these advances to the full, we must pursue the search for the many latent cases of diabetes on a much greater scale than has hitherto been the case

REFERENCES

1 ARMSTRONG, D B, DUBLIN, L I, BONNET, E C and MARKS, H L, *Postgrad Med*, 10, 407 (1951)
2 BLOTNER, H, *J Amer med Ass*, 131, 1109 (1946)
3 BORNSTEIN, J and LAWRENCE, R D, *Brit med J*, 1, 732 (1951)
4 BRINKMAN, G L, *N Z med J*, 49, 492 (1950)
5 DAHLBERG, G, *Acta paediat*, *Stockh*, 38, 91 (1949)
6 FORD, M J and GLENN, B, *J sth med Ass*, *Shreveport, La*, 44, 239 (1951)
7 HANSSEN, P, *Acta med scand*, 125, Suppl CLXXVIII (1946)
8 HARRIS, H, *Ann Eugen*, *Lond*, 14, 293 (1949)
9 HARRIS, H, *Ann Eugen*, *Lond*, 15, 95 (1950)
10 HARRIS, H, *Proc R Soc Med*, 44, 913 (1951)
11 HARRIS H and MCARTHUR, N, *Ann Eugen*, *Lond*, 16, 109 (1951)

Hereditary obesity in mice is also associated in several cases with hyperglycaemia and glycosuria (19) Thus we are enabled to study in more detail the connection between obesity and diabetes

...son with a slight shortage
a moderate rise in the
This rise in blood sugar
may promote his appetite and so lead to obesity As the disease progresses, it becomes manifest diabetes, and by now the patient has already become fat

HEREDITY

During the last couple of years, Harris of the Galton Laboratory has published a series of studies of diabetes and heredity (8, 9, 10, 11) These studies are certainly the best so far, with regard to both genetics and statistics, yet they leave several important questions unanswered

In young diabetics, Harris has found an increase in the incidence of parental consanguinity, but no increase was detected in those diabetics in which the disease developed later in life (8)

Among 3827 siblings of 1241 diabetics, there were 166 (43 4 per mille) who also suffered from diabetes (9) Those diabetics *with siblings suffering from diabetes beginning before* the age of thirty were themselves much oftener subject to diabetes early in life when they were compared with diabetics whose siblings developed the disease after the age of thirty *Among 2482 parents* of diabetics there were 125 (50 3 per mille) who themselves
... correlation between
The incr-
was below
10 per mille

These investigations by Harris seem to indicate that diabetes *is* not a genetic entity The cases of diabetes beginning in young persons and the cases beginning later in life must have their origin

observations scattered through the literature in addition to the examples which I have mentioned in my paper

LAWRENCE Wilder produced evidence a year or two ago that the idea of anticipation was not correct

HOET I might draw your attention to a paper by Katsch, who studied six cases of identical female twins When one of the twins became pregnant it developed diabetes years earlier than the other, although it, too, became diabetic eventually (KATSCH, G, *Zbl Gynak*, 72, 1756, 1950)

LAWRENCE I was never impressed by the theory that diabetes is inherited in a completely recessive manner Twenty years ago I collected 400–500 pedigrees (mainly from upper class families) When the pedigrees were shown to Hogben he said they indicated the presence of two dominant genes, Haldane on the other hand, said there was no proof So one cannot reach any conclusion There is no doubt, however, that there is a hereditary factor Harris' work is very important, he shows that although there are exceptions, the fat and thin types are genetically different This means that there is not a uniform genetic pattern There is a genetic background in forty per cent of my patients, but the underlying mechanism of inheritance is still unknown

To turn again to the difference between the fat and the thin types—there is usually a stage of about one year before the development of diabetes in the thin type in which 10–16 lb weight is gained In the early stage of the disease there is more often than not some obesity which disappears later on

LONG I am greatly impressed by the association there seems to be between obesity and diabetes The great majority of diabetics are overweight prior to the onset of the disease There may be genetic factors in the background, but I feel that the core of the problem is the influence of supranormal weight Even animals as resistant as rats if made obese [illegible]
of [illegible]

LONG Yes, I do

LUKENS When animals with a very mild pituitary diabetes are made [illegible]

[illegible] diabetes

LAWRENCE The disputes about the normal blood sugar curves which should be taken as standard are not easy to settle Early diagnosis is very helpful We would like to take a set of new diabetic

12 HARTING, D and GLENN, B, *New Engl J Med*, 245, 48 (1951)
13 JOSLIN, E P, *J Amer med Ass*, 115, 2033 (1940)
14 JOSLIN, E P, *The Treatment of Diabetes Mellitus*, 8th ed, Philadelphia Lea & Febiger (1946)
15 KENNY, A J, CHUTE, A L and BEST, C H, *Canad med Ass J* 65, 233 (1951)
16 LANCASTER, H O, *Med J Aust*, 1, 117 (1951)
17 LONG, C N H, *Diabetes*, 1, 3 (1952)
18 MCLOUGHLIN, C J, PETRIE, L M and FETZ, R H, *J med Ass, Ga*, 40, 285 (1951)
19 MAYER, J, BATES, M W and DICKIE, M M, *Science*, 113, 746 (1951)
20 SMILLIE, W G, *Public Health Administration in the United States*, 3rd ed, New York Macmillan (1947)
21 THOMPSON, M W, LAAKSO, L E and WATSON, E M, *Canad med Ass J*, 63, 556 (1950)
22 WATSON, E M and THOMPSON, M W, *Amer J dig Dis*, 18, 326 (1951)
23 WHITE, P and PINCUS, G, in JOSLIN, E P, *The Treatment of Diabetes Mellitus*, 8th ed, p 56 Philadelphia Lea & Febiger (1946)
24 WILDER, R M, *Clinical Diabetes Mellitus and Hyperinsulinism* p 53, Philadelphia W B Saunders Co (1940)
25 WILKERSON, H L C and KRALL, L P *J Amer med Ass*, 135 209 (1947)

DISCUSSION

YOUNG Harris concluded that diabetes is not a single genetic entity Does this mean that there is a genetic background with other factors superimposed?

HANSSEN Yes Harris also believes that the disease which occurs in young diabetics may be inherited homozygously and that in older diabetics in a heterozygous manner

CONN Is there not a relation between the two? The fact that a child of parents who developed mild diabetes in middle age may itself develop the disease quite early in life indicates that there probably is a connection

HOET Have you any further comments to make, Dr Hanssen on the anticipation of diabetes, that is, the occurrence of the disease in a child at an earlier age than in the parents?

HANSSEN I have no further comments to add There are some

I should be interested to hear how the answer to this question is put before the public in Norway

HANSSEN We say in Norway that early diagnosis may be decisive in ensuring that the treatment has a beneficial effect, and that early treatment may sometimes result in an apparent reversal of the disease

An increase in the caloric intake markedly affects the blood sugar level A patient taking in 2000 kg cal per day had a normal fasting

required insulin

LONG We obtained exactly the same curves with the rats which had been made obese as one normally observes with diabetes The rats were partially depancreatized to the extent at which there was more or less no sugar in the urine They were then made obese by provoking hyperphagia with suitable hypothalmic lesions The food intake was increased and glycosuria developed Two or three weeks later the food intake was restricted to the pre-operative level, but the glycosuria did not return to the level existing before induction of obesity Further periods of unrestricted food intake caused further impairment of insulin secretion as judged by the increase in the amount of glucose excreted on a restricted diet

YOUNG Is the downward trend in the diabetic mortality rate which was evident in Great Britain during the recent war, still continuing?

HANSSEN The downward trend of the diabetic mortality rate in Great Britain in the years 1914–1918 was also seen in Norway But it is difficult to compare the figures for the earlier years with those for the last few years as different methods are now used for classification of the mortality rate

YOUNG What is the ratio of female to male diabetics now? I believe there has been a great change in Great Britain over the last fifty years.

HANSSEN The ratio in the years 1915–1920 was approximately 1 0, but in the period 1920–1945 it fell to between 0 5 and 0 6 Similar changes have been noted for Canada and other countries for which reliable statistics are available The change may be correlated with the increased survival rate of elderly women As most of these old women are obese there may be a dependence on better feeding

LAWRENCE In Germany in 1918 the incidence of the fat type of diabetes was almost nil, but it has now returned The change in Great Britain since 1920 is almost certainly due to the fact that there is now abundant food for everybody The fattening of women in middle age is probably the cause of the increase of the female to male ratio for new diabetics

LUKENS You have discussed how to detect diabetes Dr Hanssen But it is important to be able to say why you want to detect diabetes

I should be interested to hear how the answer to this question is put before the public in Norway

HANSSEN We say in Norway that early diagnosis may be decisive in ensuring that the treatment has a beneficial effect, and that early treatment may sometimes result in an apparent reversal of the disease

TYPE III LIPOATROPHIC DIABETES

This is another extremely rare type of diabetes of which I described one case fully, or as far as my observations went, in 1946 (2) It is characterized primarily by the failure to have fat in any of the usual depots, subcutaneous and retroperitoneal This year another similar case was referred to me from Bristol by the kindness of Professor Neale who first recognized her condition, and this removes my slight doubts that this is not a freak but an established syndrome It is characterized by lack of [illegible]

hyperlipaemia whenever the glycaemia is high General health remains good, but an enlarged liver and portal cirrhosis develops and is ultimately lethal An extremely high metabolic rate is present without any thyrotoxicosis The whole syndrome presents to me an inexplicable picture, but I have put forward the explanation that the inability to store fat prevents the usual end-action of insulin and so produces the 'diabetic' state And this leads to the opposite state in lipoplethoric diabetics, overloaded with fat stores, who also cannot readily store circulating ingested carbohydrate and so incur diabetes

Further speculation to explain both lipoatrophic and lipoplethoric diabetes would seem to postulate an unknown enzyme or hormonal factor regulating fat deposition In this I introduce an idea for your discussion and future experimentation on which I have no clear ideas Professor Gray's department at King's College Hospital seems to have found a new steroid by paper chromatography, but full investigation has been handicapped by lack of the patient's co-operation

EXPERIMENTAL WORK

We made an attempt at King's College Hospital to prove the existence of these clinical types by animal experiments when Dr Bornstein joined us with a new technique for plasma insulin estimation using rats (1) These rats (ADHA rats) are first made diabetic with alloxan, then hypophysectomized and bilaterally totally adrenalectomized—a preparation difficult to prepare,

maintain and standardize. However, injections of between 50 and 500 microunits of insulin showed satisfactory quantitative hypoglycaemic effects. After such standardization, the fundamental test consisted of the injection of 1 ml of rapidly separated heparinized human plasma from different types of diabetic patients and the observation of any changes over one hour in the blood sugar concentrations of these rats. The early results are shown in Tables I and II.

TABLE I

Blood insulin level in type I diabetics (severe with ketosis)

Patient	1	2	3	4	5
Sex and age	M 32	F 52	F 9	M 28	M 42
Weight (kg)	46	56	25	41	54
Blood sugar content (mg per cent)	431	382	406	268	318
Glycosuria	++++	++++	++++	++++	++++
Ketonuria	++++	+	++++	+	++
Mean change in blood sugar content	+1	−3	−3	0	−2
Standard deviation	±6	±9	±7	±6	±7
Plasma insulin level (milliunits ml.)	0	0	0	0	0

0 = below lower limit of the assay

TABLE II

Blood insulin level in type II diabetics (obese no ketosis)

Patient	6	7	8	9	10
Sex and age	M 35	F 47	F 53	F 46	M 57
Weight (kg)	79	69	75	85	83
Glycosuria	++++	++++	+++	++++	++++
Ketonuria	0	0	0	0	0
Mean change in blood sugar content	−25	−30	−22	−25	−23
Standard deviation	±12	± 9	± 6	± 8	± 8
Plasma insulin level (milliunits ml.)	0.26	0.29	0.19	0.24	0.20

Other interesting results concern insulin resistance, the subsequent insulin resistance created by administration of plasma from Type I diabetics, acromegaly and other perplexing problems

REFERENCES

1 BORNSTEIN, J and LAWRENCE, R D, *Brit med J*, 1 732 (1951)
2 LAWRENCE, R D, *Lancet*, 1 724 773 (1946)
3 LAWRENCE, R D, *Brit med J*, 1, 373 (1951)

DISCUSSION

LONG The explanation suggested by Dr Lawrence for the occurrence of the lipoplethoric type of diabetes was that the fat depots are so overloaded that no effect of insulin on fat deposition can occur But if such patients are given insulin the blood sugar level falls This [illegible]

the ketonuria of two cases of type I at ++++ and of two cases at + Is a qualitative test for ketones adequate to distinguish between the different types of diabetes? Further, are not blood ketones even more important than urinary ketones? Very little is known about the urinary threshold for ketones in a normal man Our experiments with rats indicate that the blood ketone level may rise to 20 mg per cent before an appreciable ketonuria develops I suggest therefore that other more quantitative methods would give more useful information

LUKENS This question is essentially the same as that put by Dr Long is the difference between types I and II one of degree rather than kind? There may also be some differences of kind *within* the lipoplethoric type

LAWRENCE Unless the patient is in coma or has a kidney block the blood ketone level runs parallel to that of the ketones in the urine The Rothera test will detect one part in 400 000 in the urine the ferric chloride test one part in 1000

LONG But a large proportion of the ketones is β-hydroxybutyric acid which is not estimated by this method

LAWRENCE About seventy per cent is β-hydroxybutyric acid twenty per cent acetoacetic acid and ten per cent acetone

LUKENS Is the loss of fat in the lipoatrophic type due to hyperthyroidism which also produces the diabetes, and not *vice versa*?

LAWRENCE In the case of the first woman I described which had

type III diabetes, the high basal metabolic rate was not associated with clinical thyrotoxicosis, and after the thyroid had been removed she was still in the same state

[illegible]

native criteria can be used

LAWRENCE On this question of insulin sensitivity, I agree with Dr Long that insulin may produce a fall in the blood sugar level in cases of type II They also need much more insulin than those of type I But can we say what this means?

Regarding Dr Lukens' point about action in the tissue—the effects of insulin are always seen in the tissue.

LAZAROW Do you have any figures for the insulin content of the blood of non-diabetic patients who have ketosis? It is important to know the insulin level in cases of this type Since all the diabetic patients which had a low blood insulin level also had ketosis, I am wondering whether the low blood insulin level is related to the ketosis

LAWRENCE We have not made any determinations on non-diabetics with ketosis The blood insulin assays are difficult to do because of the difficulty of preparation of the ADHA rats used for the estimations Bornstein showed that the blood insulin level during fasting is lower

[illegible]

With regard to the relation of obesity to diabetes—it is worth mentioning that obesity is not always a result of overeating It has been shown that some fat people have a higher respiratory quotient than others after a carbohydrate meal, and it agrees well herewith that obese persons have a lower fasting respiratory quotient than normal persons after carbohydrate intake This indicates that in the obese persons a proportionately greater part of the carbohydrate in the food is converted into fat before combustion According to what we have recently learnt, this may well mean that there is a greater claim on insulin production in the pancreas

LAWRENCE I admit that obesity is not always due to overeating but may be due to a difference in the basal metabolic rate But one must keep in mind the first law of thermodynamics I agree with Dr Hagedorn and Dr Lukens that in type II there may be many subtypes My scheme is merely provisional and has been produced in an attempt to sort out the main types of diabetes mellitus I hope some-

body will be able to produce an experimental animal which has no fat stores, though I do not know how it will be done

LONG With the same amount of food, Dr Hagedorn will one person get ketosis and another not?

HAGEDORN Yes, the rates of accumulation may differ

LONG Carbohydrate may be either utilized or stored as fat If the latter occurs, insulin will be required The amount of carbohydrate in the diet which is converted to fat is therefore important and will depend upon the energy expenditure Obesity will be determined by the balance between intake and expenditure of energy

LAWRENCE The work of Wrenshall on the estimation of insulin in the pancreas by extraction and by the beta-cell staining method indicates that a very low value say ten per cent of the normal (which would lead to diabetes in animals), only occurs in 'growth period diabetes, that is, in those cases below the age of twenty-two If the diabetes occurs above the age of twenty-two there may be fifty per cent of the normal amount of insulin determinable by these methods Our findings are not strictly correlatable with age Type II occurs in middle age, but type I may occur at any time during the life span

WILHELMI Has Dr Lawrence anything to say about the very large liver in the lipoatrophic case?

LAWRENCE This was due to ordinary portal cirrhosis

CONN I have been trying to explain to myself the puzzling circumstance that excessive oxygen consumption occurred in the absence of hyperthyroidism or other clinical causes of a greatly increased oxygen consumption Most of us do not believe that fat is converted to carbohydrate, at least to any important extent If in Dr Lawrence's lipoatrophic diabetic a special situation existed in which body fat began to disappear because it was being converted to carbohydrate, much more oxygen would be required to accomplish this conversion This could entirely account for this peculiar syndrome If this is the explanation, the respiratory quotients should have been very low Were careful measurements made, Dr Lawrence?

LAWRENCE Many The values ranged from 0 69 to 0 85 in the fasting state as in all cases of diabetes I have no further explanation to offer

WILHELMI Some recent unpublished work of Bernheim shows that there may be in tissues an aspect of the metabolism of fatty acids which acts as an autoregulator of tissue oxidation It seems that certain fatty acid peroxides can act as modulators This mechanism may be absent in the above case

Also, in the absence of subcutaneous fat, the insulation of the skin

against heat loss is probably very different. The change in basal metabolic rate may therefore be due to a greater loss of heat

CONN Then there should be shivering

LAWRENCE This was not seen as the liver was enormous and this is where most of the heat production occurs I might add that the thyroid was quite normal in histology, as were all the endocrine glands

body will be able to produce an experimental animal which has no fat stores, though I do not know how it will be done.

LONG With the same amount of food, Dr Hagedorn, will one person get ketosis and another not?

HAGEDORN Yes, the rates of accumulation may differ.

LONG Carbohydrate may be either utilized or stored as fat If the latter occurs, insulin will be required The amount of carbohydrate in the diet which is converted to fat is therefore important and will depend upon the energy expenditure Obesity will be determined by the balance between intake and expenditure of energy

LAWRENCE The work of Wrenshall on the estimation of insulin in the pancreas by extraction and by the beta-cell staining method indicates that a very low value, say ten per cent of the normal (which would lead to diabetes in animals), only occurs in 'growth period' diabetes, that is, in those cases below the age of twenty-two If the diabetes occurs above the age of twenty-two, there may be fifty per cent of the normal amount of insulin determinable by these methods Our findings are not strictly correlatable with age Type II occurs *in middle age, but type I may occur at any time during the life* span

WILHELMI Has Dr Lawrence anything to say about the very large liver in the lipoatrophic case?

LAWRENCE This was due to ordinary portal cirrhosis

CONN I have been trying to explain to myself the puzzling circumstance that excessive oxygen consumption occurred in the absence of hyperthyroidism or other clinical causes of a greatly increased oxygen consumption Most of us do not believe that fat is converted to carbohydrate, at least to any important extent If in Dr Lawrence's lipoatrophic diabetic a special situation existed in which body fat began to disappear because it was being converted to carbohydrate, much more oxygen would be required to accomplish this conversion This could entirely account for this peculiar syndrome If this is the explanation, the respiratory quotients should have been very low Were careful measurements made, Dr Lawrence?

LAWRENCE Many The values ranged from 0 69 to 0 85 in the fasting state, as in all cases of diabetes I have no further explanation to offer

WILHELMI Some recent unpublished work of Bernheim shows that there may be in tissues an aspect of the metabolism of fatty acids which acts as an autoregulator of tissue oxidation It seems that certain fatty acid peroxides can act as modulators This mechanism may be absent in the above case

Also, in the absence of subcutaneous fat, the insulation of the skin

against heat loss is probably very different The change in basal metabolic rate may therefore be due to a greater loss of heat

CONN Then there should be shivering

LAWRENCE This was not seen as the liver was enormous and this is where most of the heat production occurs I might add that the thyroid was quite normal in histology, as were all the endocrine glands

A STUDY OF THE DIABETOGENIC ACTION OF PREGNANCY

By

J P HOET

Cliniques Universitaires St-Pierre, Louvain, Belgium

INTRODUCTION

The study of the relationship between pregnancy and diabetes falls into two distinct periods of time the first covering clinical observation of diabetic patients treated by diet, before the discovery of insulin, and the second period being characterized by the study of pregnancies in patients treated with insulin

The fact that since 1924 a large number of pregnant diabetics have been treated with insulin has revealed that there is a very high foetal mortality rate in spite of a considerably more favourable prognosis and regular and adequate treatment Moreover, this neonatal mortality and the loss of the foetus during intra-uterine life precedes the appearance of permanent glycosuria by several years, prediabetic pregnancies are characterized by foetal gigantism and appear ten to fifteen years before permanent glycosuria and clinically manifest diabetes

The experimental problem presents itself in a rather different manner Carlson, Lafon and Aron and their collaborators (cited in 30) made observations on pancreatectomized bitches to see whether the hormone from the islets of Langerhans in the foetus could cross the placental barrier The resistance to pancreatectomy at the terminal period of pregnancy has not been definitely established Markowitz and Soskin in particular (25) were not able to confirm the experimental results of Carlson on the bitch, a bitch pancreatectomized during the last weeks of gestation incurred diabetes of the same degree of gravity as that caused by operation on a non-pregnant animal

Ivy and his collaborators (cited in 30) were able to show with bitches rendered diabetic by pancreatectomy and kept in equilibrium by injection of insulin that pregnancy has a poor insulin-sparing effect This amounts to not more than 25 per cent and seems to depend on the number of foetuses, the insulin-sparing effect is only noticeable when there are more than two Ivy and

his collaborators considered that the utilization of carbohydrate in the rapid development of the foetuses was the most probable explanation We cannot dwell on this problem, since its interpretation is certainly complex, but it seems to us improbable that there is a passage of foetal insulin into the mother s system It is evident however in the diabetic woman as well as in the pancreatectomized bitch, that parturition is followed by a period during which hypoglycaemic symptoms tend to appear (21 24)

Clinical experience has shown that there is a reduction of variable degree in the insulin requirement of the diabetic patient after confinement Sometimes this sensitivity to insulin during the postpartum period is more marked We cannot share the oversimplified interpretation that this is due exclusively to the onset of lactation In fact although this condition is provoked by parturition it continues for several weeks or sometimes months This reaction is characteristic of certain cases For example, I have reported the case of a patient who had been suffering from diabetes for several years after delivery the insulin dose, which had varied between 20 and 30 units, had to be reduced to 4 units [illegible]

but we believe that it is connected on the one hand, with the increased production of adrenal corticosteroids during gestation, and on the other hand with the reduction in the amount of corticosteroids secreted after parturition The level of corticosteroids in the blood of the pregnant woman would be a much more direct indication than the amount in the urine which was [illegible]

syndrome The adrenal cortex secretes progressively less hormone until the secretion becomes normal some weeks after parturition This demonstrable secretion from the adrenals constitutes a physiological diabetogenic element whose effect may last for a period of several months

The clinical data concerning the prediabetic state may be

A STUDY OF THE DIABET(OF PREGNA

By

J P HO

Cliniques Universitaires St P

INTRODU

The study of the relationship l falls into two distinct periods of observation of diabetic patients covery of insulin and the seco the study of pregnancies in pa

The fact that since 1924 a l have been treated with insul high foetal mortality rate in s able prognosis and regular this neonatal mortality and uterine life precedes the app several years prediabetic p gigantism and appear ten to suria and clinically manife

The experimental probl manner Carlson Lafon in 30) made observatio whether *the hormone fr* could cross the placental l at the terminal period ol lished Markowitz and confirm the experimen pancreatectomized du diabetes of the same d on a non-pregnant a

Ivy and his collab bitches rendered dia brium by injection (sparing effect This seems to depend o effect is only notice

several months afterwards concluded that in 80 per cent of the

of glucose in pregnant women (7)

Selman reports that of forty-seven pregnant women twenty-two gave a hyperglycaemic curve too high or too prolonged In individual cases it is easy to observe the very marked influence of pregnancy on the form of the hyperglycaemia curve We reproduce the graph given by Hurwitz and Jensen (15) (Fig 1) and

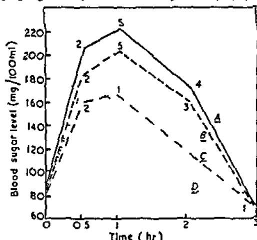


Fig 1

Glucose tolerance curves for the same patient at various times during pregnancy and after delivery (from ref. 15)

A, second trimester *B* third trimester *C* one month postpartum *D* eight months postpartum

Degree of urine sugar reduction 1 green without precipitate 2 green with precipitate 3 olive 5 brick-red

also th

interpreted in the light of the physiological data of pregnancy An account is therefore given later in this paper of P L Hoet's experimental study of the effect of cortisone during gestation, and in particular the first results concerning its effect on placental glycogen

THE INTERPRETATION OF HYPERGLYCAEMIC CURVES DURING PREGNANCY

Glycosuria during pregnancy has often been considered as

of intravenous injections of glucose, have been compared at *various stages of pregnancy and independently of pregnancy*, the conclusions have been variable It appeared desirable in initiating this investigation to compare the averages of certain groups Indi-

levels *during glucose tolerance tests* carried out on pregnant subjects, we quote the results of certain investigations

Johnson and Bousnes summarize their observations as follows 'Glucose injected intravenously disappears from the blood of the pregnant patient at the same rate as from the blood of the patient who is not pregnant There is a tendency for glycaemia to fall to the level of fasting glycaemia more rapidly in the pregnant woman than in the woman who is not pregnant' (18) Hurwitz and Jensen (15) confirm the observations of Labbe and Chevki (20) that during pregnancy all degrees of glycoregulation dis- … be found that

hat

ing

es-

sive pregnancy Labbe and Chevki considered that preg… cy, *and especially multiple pregnancies, could* sometimes be the origin of diabetes Hurwitz and Jensen, from observations of twenty-five normal pregnant women made (a) at three-monthly intervals during pregnancy, (b) immediately after parturition, and (c)

with an anterior pituitary extract containing the diabetogenic-growth complex In alloxanized rats there were 18 per cent of stillbirths and 19 per cent of neonatal deaths Moreover 50 per cent of the offspring which were born alive died before weaning, whilst the anterior pituitary extract produced 100 per cent stillbirths Chorionic gonadotrophin also gave rise to a very high foetal mortality rate

In spite of the interest of these experiments the pathogenic role of growth hormone is difficult to evaluate, since it cannot be estimated either in the blood or in the urine It is not known how when or in what quantity the growth hormone is secreted It may even be denied that this hormone plays a diabetogenic role during pregnancy Indeed Young has noted in several instances that growth hormone does not provoke diabetes in pregnant bitches (42) This fact was observed in one of the first animals which Young injected with growth hormone for a prolonged period On the other hand growth hormone reduces the blood sugar level in fasting rats

A STUDY OF THE EFFECTS OF CORTISONE ON THE PLACENTAL GLYCOGEN OF PREGNANT RABBITS

We have investigated the effects of administering cortisone during pregnancy, in the first place because there are considerable modifications in the excretion of gluco-corticosteroids during pregnancy (5, 37) and the concentration of 17 hydroxycorticosterone in the serum is definitely higher during the last three months of … ortisone … ges em- … weight per day for six days) resulted in resorption of the foetus We have related this study to that of the changes in the placental glycogen which occur under the influence of cortisone

Placental glycogen does in fact appear to play a role of prime importance in the regulation of foetal nutrition Claude Bernard

are very closely interrelated The results of Paton (31) and Kriss and Futcher (19) give sufficient indication of this Attention is drawn to Table II, taken from a paper by Paton (31), which consists of one group comprising all abnormal pregnancies (miscarriage, prematurity, foetal gigantism, death during delivery and neonatal mortality), and a second group in which miscarriages resulting from accidents of pregnancy are given These two groups are arranged according to the number of years by which pregnancy preceded the appearance of permanent glycosuria In brief, miscarriages were observed to occur up to twenty-five years before the appearance of diabetes, foetal gigantism occurs forty years before the discovery of permanent glycosuria, and prematurity, death at birth and neonatal mortality occur thirty [illegible]

[illegible]

the foetus survives, but in which the newborn infant is excessively heavy The next most serious form is that which leads to death *in utero* or during extrauterine existence, but where the pregnancy continues until full term, or nearly so The most serious form is that characterized by miscarriage

Although we cannot give many statistics, we stress the fact that the use of insulin, even in small doses, modifies the prognosis of these pathological pregnancies, which are characterized by glucose tolerance tests showing transitory changes during pregnancy

The pathogenic problem has been closely studied, both clinically and experimentally, and the following main points may be presented

The histological reactions of the pituitary lead us to suspect a hypersecretion by the eosinophil cells Watts showed in 1935 that administration of growth hormone to rats can cause the foetuses to be of excessive weight, but can also cause death (38) Hullquist and Engfeldt, and Barns and his associates have also produced [illegible] growth hormone, as well

[illegible] i spite of

[illegible] wth hor-

mone in rats can cause death *in utero* and even a certain increase in weight of the foetus, the experiments have no relation to carbohydrate metabolism

Barns *et al*, in their work on pregnant rats, demonstrated the very great sensitivity of the hormonal equilibrium which ensures the nutrition of the foetus (3) They compared the foetal mortality rate of alloxan diabetic rats with that of pregnant rats injected with an anterior pituitary extract containing the diabetogenic-growth complex In alloxanized rats there were 18 per cent of stillbirths and 19 per cent of neonatal deaths Moreover, 50 per cent of the offspring which were born alive died before weaning, whilst the anterior pituitary extract produced 100 per cent stillbirths. Chorionic gonadotrophin also gave rise to a very high foetal mortality rate

In spite of the interest of these experiments, the pathogenic role of growth hormone is difficult to evaluate, since it cannot be estimated either in the blood or in the urine. It is not known how, when, or in what quantity the growth hormone is secreted It may even be denied that this hormone plays a diabetogenic role during pregnancy Indeed, Young has noted in several instances that growth hormone does not provoke diabetes in pregnant bitches (42) This fact was observed in one of the first animals which Young injected with growth hormone for a prolonged period On the other hand, growth hormone reduces the blood sugar level in fasting rats

A STUDY OF THE EFFECTS OF CORTISONE ON THE PLACENTAL GLYCOGEN OF PREGNANT RABBITS

We have investigated the effects of administering cortisone during pregnancy, in the first place because there are considerable modifications in the excretion of gluco-corticosteroids during pregnancy (5, 37) and the concentration of 17-hydroxycorticosterone in the serum is definitely higher during the last three months of pregnancy (4), secondly, because ACTH and cortisone consistently provoke miscarriage (6, 12 33) The dosages employed (which varied between 10 and 25 mg per kg body weight per day for six days) resulted in resorption of the foetus We have related this study to that of the changes in the placental glycogen which occur under the influence of cortisone

Placental glycogen does in fact appear to play a role of prime importance in the regulation of foetal nutrition. Claude Bernard

are very closely interrelated The results of Paton (31) and Kriss and Futcher (19) give sufficient indication of this Attention is drawn to Table II, taken from a paper by Paton (31), which con-

groups are arranged according to the number of years by which pregnancy preceded the appearance of permanent glycosuria In brief, miscarriages were observed to occur up to twenty-five years before the appearance of diabetes, foetal gigantism occurs forty years before the discovery of permanent glycosuria, and prematurity, death at birth and neonatal mortality occur thirty years before its appearance

Paton establishes a progression (31) which it is worth our while to note The least serious form of the condition is that in which the foetus survives, but in which the newborn infant is excessively heavy The next most serious form is that which leads to death *in utero* or during extrauterine existence, but where the pregnancy continues until full term, or nearly so The most serious form is that characterized by miscarriage

Although we cannot give many statistics, we stress the fact that the use of insulin, even in small doses, modifies the prognosis of these pathological pregnancies, which are characterized by glucose

presented

to be of excessive weight, but can also cause death (38) Hultquist and Engfeldt, and Barns and his associates have also produced

mone in rats can cause death *in utero* and even a certain increase in weight of the foetus, the experiments have no relation to carbohydrate metabolism

ve showed a definitely greater hyper-
n of 0 5 mg cortisone per kg in pregnant
gnant rabbits

CONCLUSIONS

considerable further examination but they

ACTH and cortisone previously described
and Collonge and Robson and Sharaf (6
ons also emphasize the very close relation-
ional condition of the islets of Langerhans
which was clearly described by Long and
recently been studied afresh by Thorn,
(35)
adition which constitutes an important
y of pregnancy, may also be studied from
int of view
glycosuria recurs in pregnancy, injections

nal equilibrium of the foetus in relation to
il glycogen level will necessarily lead to a
edge of the toxaemias of pregnancy
is in the serum by the methods at present
Vogt Bush Nelson and Samuels and
ates, may show not only that pregnancy is
that the hormonal balance which exists
ls to increase resistance to insulin Many
e shown to have a functionally deficient
of the mother during pregnancy may con-

onus Newborn infants will also be pro-
pathological investigations can lead to a

curve showed a definitely greater hyper-
ection of 0 5 mg cortisone per kg in pregnant
pregnant rabbits

CONCLUSIONS

heed considerable further examination but they

on of ACTH and cortisone previously described
ner and Collonge and Robson and Sharaf (6
vations also emphasize the very close relation-
unctional condition of the islets of Langerhans
tex which was clearly described by Long and
has recently been studied afresh by Thorn
all (35)
condition which constitutes an important
logy of pregnancy may also be studied from
l point of view
t if glycosuria recurs in pregnancy injections

itional equilibrium of the foetus in relation to
ental glycogen level will necessarily lead to a
owledge of the toxaemias of pregnancy
roids in the serum by the methods at present
by *Vogt Bush Nelson and Samuels* and
sociates may show not only that pregnancy is
lso that the hormonal balance which exists
tends to increase resistance to insulin Many
n be shown to have a functionally deficient
us of the mother during pregnancy may con-
ed by safeguarding the controlling action of
hand by injection of insulin and on the other
pocaloric diet and possibly also by terminating
months Newborn infants will also be pro-
siopathological investigations can lead to a

Table IV

Effects of cortisone on foetal we ght and placental glycogen level in pregnant rabbits

(M P maternal placenta F P foetal placenta F L, foetal liver)

No of rabbit	Weight (kg)	Foetal age (days)	Dose of cortisone (mg/kg/day for 4 days)	Foetuses	No of foetus I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
51	3 30	16–19	0 5	Weight (g)														
				Foetus	2 10	2 95	2 99	2 65		2 76	2 46	2 81	2 80	2 99	3 11	2 72		
				M P	1 07	1 20	0 95	1 99		1 34	1 12	1 00	1 02	1 47	1 61	1 46		
				F P	1 24	1 38	1 45	1 52		1 60	1 15	1 14	1 40	1 24	1 53	1 57		
				Glycogen content (g/100 g)														
				M P	0 475						0 458							
				F P	0 288						0 262							
				F L	0 187						0.120							
52	3 10	19	0	Weight (g)														
				Foetus	2 25	2 15	2 60	2 65	2 50	2 08	2 15							
				M.P	1 21	1 02	0 95	1 49	1 29	1 22	1 26							
				F P	1 43	1 30	1 34	1 48	1 28	1 21	1 99							
				Glycogen content (g/100 g)														
				M P		0 81	0 87											
				F P	0 298	0 25	0 32											
				F L	0 127	0 18	0 11											

55	3.00	14	0.5	Weight (g)														
				Foetus	0.18	0.23	0.16	0.13	0.20	0.19	0.24	0.22						
				M P					0.96	0.97	1.18	1.29						
				F P					0.45	0.51	0.38	0.54						
				Glycogen content (g/100 g)														
				M P	5.25	5.78	6.24											
				F P	1.45	1.48	2.70											
56	3.00	14	0.5	Weight (g)														
				Foetus	0.30	0.25		0.25	0.28	0.23	0.18	0.28	0.30	0.25	0.26	0.30	0.26	0.25
				M P				1.15	1.32	1.65	1.25	0.25	1.25	1.43	0.85	1.35	1.07	0.90
				F P				0.45	0.46	0.55	0.43	0.45	0.78	0.68	0.75	0.67	0.90	0.45
				Glycogen content (g/100 g)														
				M P	4.70	4.98												
				F P	2.50	1.85	1.81											
28	2.20	16–17	0.5	Weight (g)														
				Foetus	2.00	2.35												
				M P	0.75	0.70		0.75	0.53	0.85	0.93	0.79						
				F P	0.81	0.71		0.90	0.82	1.23	0.60	0.66						
				Glycogen content (g/100 g)														
				M P	5.60					6.5	5.82							
				F P	0.49						0.42							
				F L	0.17													

fuller understanding of the characteristic pathology of the living infant born of a diabetic or prediabetic mother

SUMMARY

1 The end of pregnancy and the post-partum period are characterized by a tendency to hypoglycaemia and a marked decrease in the need for insulin This is in contrast to the increased need for insulin, endogenous or exogenous, which arises during the course of pregnancy

[illegible]

severe during successive pregnancies

3 Miscarriage may be induced experimentally in pregnant rabbits by injection of 2 to 5 mg cortisone per kg body weight per day In doses of 0 5 mg per kg per day, cortisone causes a rapid increase in foetal weight Before the sixteenth day of gestation, this dose of cortisone causes an increase in the amounts of glycogen in both foetal and maternal placentas

4 From the twenty-first day of pregnancy onwards, cortisone does not cause an increase in the amount of maternal placental glycogen, but accelerates the increase in weight of the foetus

5 The hyperglycaemic effect of cortisone is greater during pregnancy

REFERENCES

1 ALLEN, E, *Amer J Obstet Gynec*, 38, 392 (1939)
2 BERNARD, C, *C.R Acad Sci, Paris*, 48, 77 (1859)
3 BARNS, H H F, LINDAN, P, MORGANS, M E, REID, E and SWYER, [illegible] *Lancet*, ii, 841 [illegible]
4 BUSH, I [illegible]
5 COPE, C [illegible] and [illegible] *it med J*, ii 762 (195[illegible]
6 C[illegible] *Ar*, 146, 493 (195[illegible]
[illegible], 67, 1433 ([illegible]

8 GILBERT, J A M and DUNLOP, B A, *Brit med J*, 1, 48 (1948)
9 HEARD, R D H and SOBEL, H, *J biol Chem*, 165, 687 (1946)
10 HERZSTEIN, J and DOLGER, H, *Amer J Obstet Gynec*, 51, 420 (1946)
11 HOET, J P, *Schweiz med Wschr*, 80, 70, 81, 420 (1950)
12 HOUSSAY, B A, *Rev Soc argent Biol*, 21, 316 (1945)
13 HUGGETT, A STG, *J Physiol*, 67, 361 (1929)
14 HULLQUIST, G and ENGFELDT, S, *Acta endocr*, *Copenhagen*, 3, 365 (1949)
15 HURWITZ, D and JENSEN, D, *New Engl J Med*, 234, 327 (1946)
16 INGLE, D J, *Endocrinology*, 28, 249 (1941)
17 INGLE, D J and FISHER, G T, *Proc Soc exp Biol N Y*, 39 149 (1938)
18 JOHNSON, D G and BOUSNES, R W, *J clin Invest*, 27, 745 (1948)

22 LOCHHEAD, G and CRAMER, W, *Proc roy Soc*, B, 80, 263 (1908)
23 LONG, C N H and LUKENS, F D W, *Science*, 79, 568 (1934)
24 MACLEOD, J J R, *Carbohydrate Metabolism and Insulin*, p 83, London, New York Longmans, Green & Co Ltd (1926)
25 MARKOWITZ, J and SOSKIN, S, *Amer J Physiol*, 79, 553 (1927)
26 MILLER, H C, *Endocrinology*, 40, 471 (1947)
27 MILLER, H C, HURWITZ, H and KUDER K, *J Amer med Ass*, 124 271 (1949)
28 MILLER, H C, JOHNSON, R D and DIERLECHER, S H, *J Pediat*, 24 603 (1944)
29 MILLER, H C and WILSON, H M, *J Pediat*, 23 251 (1943)
30 NEWTON, W H, in *Marshall's Physiology of Reproduction*, 3rd ed, ed PARKES, A S vol. 2, p 478, London, New York, Toronto Longmans Green & Co (1952)
31 PATON, I E M, *Amer J Obstet Gynec*, 56, 558 (1948)
32 PFLÜGER, E, *Pflüg Arch ges Physiol*, 93, 163 (1903) 102, 305 (1904) 111, 307 (1906)
33 ROBSON, J M and SHARAF, A A, *J Physiol*, 116, 236 (1952)
34 SNYDER, F F, *Johns Hopk Hosp Bull*, 54, 1 (1934)
35 THORN, G W, FORSHAM, P H and KENDALL, E, Jr, *The Diagnosis and Treatment of Adrenal Insufficiency*, Springfield, Ill C C Thomas (1951)
36 VERHAGEN, H and BYVOET, W F, Jr, *Acta brev neerl Physiol*, 17, 70 (1950)

fuller understanding of the characteristic pathology of the living infant born of a diabetic or prediabetic mother

SUMMARY

1 The end of pregnancy and the post-partum period are characterized by a tendency to hypoglycaemia and a marked decrease in the need for insulin This is in contrast to the increased need for insulin endogenous or exogenous, which arises during the course of pregnancy

d

-

h

erythroblastosis, cardiomegaly and hypoglycaemia This pathology is familial and characterizes most of the pregnancies of the same mother Eventually it seems to become progressively more severe during successive pregnancies

3 Miscarriage may be induced experimentally in pregnant rabbits by injection of 2 to 5 mg cortisone per kg body weight per day In doses of 0 5 mg per kg per day, cortisone causes a rapid increase in foetal weight Before the sixteenth day of gestation, this dose of cortisone causes an increase in the amounts of glycogen in both foetal and maternal placentas

4 From the twenty-first day of pregnancy onwards cortisone does not cause an increase in the amount of maternal placental glycogen, but accelerates the increase in weight of the foetus

5 The hyperglycaemic effect of cortisone is greater during pregnancy

REFERENCES

1 ALLEN, E , *Amer J Obstet Gynec* , 38, 392 (1939)
2 BERNARD, C , *C R Acad Sci , Paris*, 48, 77 (1859)
3 BARNS, H H F, LINDAN, P , MORGANS, M E , REID, E and SWYER, G I M , *Lancet*, ii, 841 (1950)
4 BUSH, I E , private communication
5 COPE, C L , BOYSEN, X and MCCRAE, S , *Brit med J* , ii 762 (1951)
6 COURRIER, R and COLLONGE, A , *J Amer med Ass* , 146, 493 (1951)
7 FRANK, E and NOTHMANN, M , *Munch med Wschr* , 67, 1433 (1920)

completely normal during pregnancy, but hyperglycaemic fits occurred just after childbirth (POMPEN, A W M, JANSEN, C A L and THONT J, *Acta med scand*, 124, 334 1946)

HANSSEN If there is an aggravation of the diabetes during pregnancy, is the increase in the number of cases in older women explained?

HOET Yes, that may be the reason

HANSSEN I have observed that there is sometimes an improvement in the diabetes during the last few months of pregnancy This may be due to the secretion of insulin by the pancreas of the foetus

Can the hypoglycaemia which occurs immediately after childbirth be ascribed to the heavy work during confinement and to the reduced food intake?

HOET No, because it may last for several weeks It seems more likely that it is due to a reduced output by the adrenals The rate of excretion of adrenal cortical steroids is very markedly lower for one to four weeks after childbirth

LUKENS Following Hench's observations have you made any studies of pregnant women with jaundice?

HOET Many textbooks describe the abortive action of jaundice during pregnancy

LAWRENCE I feel that there are many points of dubious interpretation in your paper, Professor Hoet Many of the women who get diabetes have heavy babies, stillbirths etc Probably this is the type which becomes fat and the thin type does not have large babies The question of hormone imbalance, which you consider as proven, we are very sceptical about in Great Britain I might mention Gray's recent work he found that there was no difference between the hormonal patterns in normal and diabetic pregnancies (GRAY, C H, *Ciba Foundation Colloquium on Hormonal Factors in Carbohydrate Metabolism*, p 318, 1953) In the last two years under the guidance of the [illegible] centres [illegible] into two groups, half being given the oestrogen and the remainder serving as controls On the basis of the results to date I am very sceptical about the effects of adrenal cortical hormones in diabetic pregnancy

HOET [illegible] oestrogen therapy, Priscilla White prefers to provoke childbirth at eight months

37 VENNING, E H and BROWNE, J S L, in *Progress in Clinical Endocrinology*, ed SOSKIN, S, p 198, New York Grune & Stratton (1950)
38 WATTS, R, *Amer J Obstet Gynec*, 30, 174 (1935)
39 WHITE, P, in JOSLIN, E P, *The Treatment of Diabetes Mellitus*, [illegible]
40 [illegible]
41 [illegible] G W, *Proc Amer Diabetes Ass*, 10 25 (1950)
42 YOUNG, F G, *Acta med scand*, 135, 275 (1949)

DISCUSSION

YOUNG We carried out some experiments in which growth hormone was given to pregnant animals There were no abortions and no statistically significant increases in the size of the foetuses in the number of experiments which we carried out

HOET Fouracre Barns and his associates observed one hundred per cent mortality of the foetuses following injection of pregnant rats *with anterior pituitary extract (ref 3 of my paper)*

YOUNG Not very pure growth hormone was used in the experiments you mention We were disappointed not to find an effect Miss [illegible]

2 mg of cortisone will result in death of the foetus and it might be the same with growth hormone

YOUNG But growth hormone had no such effect in our experiments with rats

DE JONGH I would not expect growth hormone to have much effect on the foetal weight in rats, Professor Young because growth of the foetus might well be independent of growth hormone.

YOUNG But Watts claimed that there was a very clear effect (ref 38 of Professor Hoet's paper) Also, in our experiments with pregnant cats and dogs, growth hormone did not have its usual diabetogenic effect It seemed possible, therefore that its action had been diverted to the stimulation of growth of the foetus but there is not yet any proof of this theory

HOET I should have mentioned the complementary work of Ingle and Houssay in which the weights of the foetuses were found to be *lower in adrenalectomized rats*

I should like to add a note also on the occurrence of diabetic pregnancy associated with adenoma of the pancreas Eight cases, one very clear cut were reported by Pompen in 1946 Two of these cases were

HOET This was not done Both Miller and Van Beek have shown that children of diabetic mothers have hearts which are very large and very rich in glycogen (ref 26, and VAN BEEK, C, *Maandschr Kindergeneesk*, 19, 56, 1951) The adrenal cortical steroids may, therefore, be playing a role

BEST Are there any data on the total caloric intake in diabetic and non-diabetic mothers in the later stages of pregnancy?

HOET Some facts have been reported by MUNRO, H B EATON, J C and GLEN, A, *J clin Endocrin* 9, 48 (1949)

LONG Do adrenal cortical steroids mobilize material in the mother and cause it to be transferred to the foetus?

HOET ACTH from the mother may produce an effect on the adrenals of the foetus which diminishes immediately after birth, or it may be that placental ACTH plays a role It seems from our experiments that the transfer of nutritional material occurs by way of glycogen in the placenta There are some cases in the literature of children from diabetic mothers in which there was haemorrhage in the adrenals during the period immediately after birth

LUKENS Does eosinopoenia occur during diabetes in pregnancy?

HOET Yes (DAVIS, M E and HULIT, B E, *J clin Endocrin*, 9, 714, 1949)

DE DUVE Van Campenhout found that cobalt does not destroy the alpha-cells of the guinea-pig's pancreas during pregnancy

To discover transient diabetes of pregnancy, the sugar tolerance curve has to be done more than once during pregnancy and particularly from the fourth month onwards In one of my cases, a heavy transient diabetes occurred during pregnancy fifteen years before full and permanent diabetes developed

LONG Perhaps stimulation of the pituitary and adrenals is a normal physiological event during pregnancy, and diabetes may arise only if other factors are operative at the same time

HOET Adrenal cortical steroids may increase the severity of the diabetes, especially if the pancreas has insufficient functional reserves The adrenal cortical steroids depress pancreatic function

CONN On the basis of present knowledge, one runs into great difficulties when an attempt is made to explain why the babies of diabetic mothers are generally larger than normal While it is true that occasionally one observes a diminishing insulin requirement during the last trimester of a diabetic pregnancy (presumably attributable to foetal insulin secretion) the usual response is a definite increase in insulin requirement This suggests that increased amounts of anti-insulin substances are being produced

We recognize the existence of two substances that increase the insulin requirement of the diabetic, namely, adrenal cortical steroids and growth hormone Could these maternal secretions, when applied to the foetus, account for its excessive weight? It would be difficult to account for increased foetal weight on the basis of excessive activity of adrenal cortical steroids because their major metabolic effect is on catabolism One might speculate that the effect is catabolic in the mother only and that as a result, increased amounts of building materials are brought to the foetus which itself escapes the catabolic influence of the adrenal steroids

If the production of excessive amounts of growth hormone accounts for the increased need for insulin during the last three months of pregnancy, it is difficult to believe that growth hormone has stimulated the secretion of insulin and that the anabolic effects of insulin are responsible for the increased weight of the foetus

What scientists need is a comparison of carcass analyses of stillborn normals with analyses of stillborns of diabetic mothers From the emotional point of view, it is obvious that such data will be difficult to obtain

LONG The adrenals, as you well know Dr Conn, are larger just before or after birth

LUKENS Have you studied the tissue composition of the foetuses Professor Hoet? Cortisone may be producing a change in proportion of the various components

establish the relation between incidence and heredity of the disease

[illegible]

discussion among friends of a common interest in the progress of the knowledge of diabetes and its ultimate benefit to the diabetic patient

CONCLUDING REMARKS

By

R D LAWRENCE

[illegible]

experiences in the last two weeks have been a perfect illustration of such a stimulating synergism

As you know, the new International Diabetes Federation held [illegible]

The latter meetings were greatly promoted by contributors from some members of this present C I O M S symposium The International Diabetes Federation will hold another congress in Europe in three years time and we do hope that a similar scientific colloquium can be arranged in an adjoining week

Perhaps—so quick is the present flow and ebb in experimental diabetes—we may by that time find ideas profoundly changed. Perhaps glucagon will have a new name, alloxan may be a new cure for diabetes, and growth hormone may be found not to exist!

CONCLUDING REMARKS

By

J P. HOET

The progressively more detailed studies of experimental diabetes have many isolated features which seem to complicate our understanding of the clinical disease In man, the situation is further complicated because acidosis and coma have practically disappeared. The discovery of insulin permitted survival of the diabetic patient to be prolonged and resulted in the prominence of the later complications of the disease

[illegible]

none' laws about diabetes, but we ought to appreciate the importance in this condition of the dynamic aspects of carbohydrate metabolism and nutrition Indeed, nutrition, or rather overnutrition, is a conspicuous factor which develops or reveals the relative or absolute insufficiency of the performance of the [illegible]

[illegible] of the islets permit [illegible] to be formulated

During the last twenty years, several types of permanent experimental diabetes have been described The one feature common to all these new forms of experimental diabetes is that the islets are damaged, by various methods, without surgery and without injury to the acinar tissue of the pancreas It is quite clear that the islets of Langerhans ought to adapt themselves to many physiological and nutritional requirements In fact they do this surprisingly well within certain limits but, nevertheless, they are often quite vulnerable The pancreas itself appears to contain a second hormone, glucagon, which may play a part in counteracting insulin hypoglycaemia

Have we hormonal or enzymic explanations of all the observed variations in the need for insulin? Certainly, further research will give us more satisfactory answers to this question and to the problem of insulin resistance It will also help us to make still better differentiation between the various types of diabetes and to

from many different species still form the background of our discussion of dynamic mechanisms

Although we may not have evolved a method for curing [illegible]

[illegible]

tion and extension of fundamental knowledge and this we have attempted to effect in our meeting here in Leiden

diabetes, such fundamental knowledge cannot fail ultimately to be of importance in the problem which has been the main interest of this meeting

Dr Hagedorn's interesting discussion of the absorption of insulin from the human subcutaneous tissues has recalled to us that the lymph may be an important route of entry into the circulation It is noteworthy that Biedl's ancient experiments on the effect of ligation of the thoracic duct have not been more thoroughly followed up

[illegible]

significance in human diabetes has also been discussed by Dr Lawrence in his inimitable manner

[illegible]

condition The complicated hormonal balances which obtain in pregnancy leave much to be explored by future investigators in this domain

We have said remarkably little about the effects of the size and composition of the diet on the production of diabetes either experimental or clinical but we have obviously had to limit our discussion to some aspects of an enormous field

In general, we have emphasized the importance of a balance between a number of hormones in the production of diabetes rather than of the activities of a single hormone The suggestion that growth hormone elicits the secretion of both insulin and glucagon from the islets of Langerhans of the pancreas is an example of this type of reasoning The idea that not only hormones, but also abnormal metabolites such as alloxan or dehydrouric acid, may induce naturally occurring diabetes, is a fascinating one which still leaves much for investigation That hormones may in part influence carbohydrate metabolism through the production of abnormal metabolites is also a possibility that we have touched upon, though not discussed thoroughly

At every stage in our meeting we have passed from the morphological to the physiological and biochemical without hesitation Professor Best's splendid colour slides of pancreatic tissues

INDEX